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ALLELIC CHARACTERIZATION AND INHERITANCE OF POTYVIRUS RESISTANCE GENES IN CUCUMBER

By

Eileen A. Kabelka

A THESIS

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ABSTRACT

ALLELIC CHARACTERIZATION AND INHERITANCE OF POTYVIRUS RESISTANCE GENES IN CUCUMBER

By

Eileen A. Kabelka

Several sources of potyvirus resistances have been identified within the cucumber (*Cucumis sativus* L.) germplasm: 'TMG-1' is resistant to zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and the watermelon strain of papaya ringspot virus (PRSV-W); 'Dina' is resistant to ZYMV; and 'Surinam' is resistant to PRSV-W. These studies demonstrate that TMG-1 and Dina also possess resistance to the Moroccan watermelon mosaic virus (MWMV). Determination of mode of inheritance, tests of allelism among sources of resistance, and tests for cosegregation of the resistances to the different potyviruses indicate that the resistances to ZYMV, MWMV and PRSV-W in TMG-1, Dina, and Surinam are due to the same, or tightly-linked gene(s). Although resistance to these potyviruses may be conferred by a single gene, variation in dominance relationships, resistance mechanisms, symptom expression, and which potyviruses are protected against, support the possibility of a tightly-linked cluster of potyvirus resistance genes.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cucumbers and other cucurbit crops are subject to severe losses due to an array of potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV) and the watermelon strain of papaya ringspot virus (PRSV-W) (Lisa and Lecoq, 1984; Purcifull et al, 1984a,b). Recently, an additional virus that infects cucurbits, the Moroccan watermelon mosaic virus (MWMV) has been identified to be a distinct member of the potyvirus group (McKern et al, 1993). Sources of resistance to several of these viruses have been identified within the cucumber germplasm (Provvidenti, 1985). Inbred lines derived from the Chinese cucumber cultivar, 'Taichung Mou Gua' (TMG) have been identified to be resistant to ZYMV, WMV, PRSV-W (Provvidenti, 1985) and recently zucchini yellow fleck virus (ZYFV) (Gilbert-Albertini, 1995). Inheritance of resistance in TMG to these potyviruses have been characterized (Provvidenti, 1987; Wai and Grumet, 1995a,b; Gilbert-Albertini, 1995). Two additional sources of resistance include resistance to ZYMV in the Dutch hybrid 'Dina'

(Abul Hayja and Al-Shahwan, 1991) and resistance to PRSV-W in the cultivar 'Surinam Local' (Wang et al, 1984).

The goals of this study are (1) determine if TMG possesses resistance to MWMV and, if so, to investigate the relationship, if any, between resistance to MWMV and the resistance to other cucurbit potyviruses, (2) determine the relationships between resistance alleles in TMG and other sources of resistance in cucumber to potyviruses and (3) determine the relationship of different potyvirus resistances to each other.

1.2 General description and taxonomy of the potyviruses

The largest and most complex virus group in the *Potyviridae* is the genus *Potyvirus* (Ward and Shukla, 1991; Shukla et al, 1994). The potyvirus group, named after its type member potato virus Y, has caused significant loss in agricultural, horticultural and ornamental crops. The potyviral genome is composed of a single-stranded, positive-sense RNA molecule of approximately 10,000 bases. The 5'-end is covalently attached to a protein VPg and the 3'-end contains a variable length polyadenylate tail. The viruses belonging to this group are flexuous and rod-shaped, 680-900 nm long and 11-15 nm in diameter. All members share the characteristic pinwheel inclusion bodies in the cytoplasm of infected cells. Although a few members can be transmitted by mites, whitefly and soil fungi, the potyviruses are predominantly transmitted by aphids in a non-persistent

manner. Most members are easily transmitted mechanically while a few can be transmitted through seed of infected plants.

An effective taxonomy for the potyviruses has been considered difficult to establish (Shukla and Ward, 1989; Ward and Shukla, 1991; Shukla et al, 1994). Assignment into the potyvirus group, in the past, has relied on characteristics such as particle morphology, host range, symptomatology, cross-protection, cytoplasmic inclusion morphology, serology and transmission mechanisms. However, due to variation within the potyvirus group and inadequacies of the traditional methods employed to identify viruses and their strains, inconsistencies exist. In an effort to provide a rational basis for potyvirus taxonomy, Ward and Shukla (1991) evaluate and identify criteria, based on molecular and phenotypic characteristics, useful to distinguish potyviral group membership and strain identification.

For membership into the potyvirus group, the most valuable molecular characteristic is the complete nucleotide sequence which indicates the number, nature and order of coding regions along with the mechanisms of transcription, translation and replication (Shukla and Ward, 1989; Ward and Shukla, 1991; Shukla et al, 1994). Coat protein sequencing is considered the next most valued characteristic as the coat protein virus product shares no significant sequence identity with the coat protein virus products of other virus groups. Considered the most valued phenotypic characteristic for

membership into the potyviruses is the appearance of pinwheel cylindrical cytoplasmic inclusions (Ward and Shukla, 1991). This cylindrical shape identifies only the potyviruses and no other virus group. The value of other phenotypic characteristics such as particle morphology and transmission mechanisms, for distinguishing potyviral membership, depends on how accurately and uniquely they reflect the group. These phenotypic characteristics, however, along with host range, symptomatology, cross-protection, and serology, are considered useful tools for diagnostic purposes.

Molecular characteristics, as valuable criteria for potyviral group membership, also provide valuable criteria to distinguish between viruses and strains (Shukla and Ward, 1989; Ward and Shukla, 1991; Shukla et al, 1994). The sequence homology between distinct viruses and strains is significantly different (distinct members range from 38-71% while strains range from 90-99%). Because of this significant sequence homology difference, gene sequencing, coat protein sequencing or simpler techniques, such as nucleic acid hybridization, high-performance liquid chromatography peptide profiling and N-terminal-targeted serology, can be useful in distinguishing viruses and strains.

From an initial reliance based on morphological, biological, and serological properties for assignment into the potyviruses, molecular approaches, such as nucleic acid and coat protein sequencing, will ultimately

lead to a more accurate and detailed system of virus identification and classification.

1.3 Occurrence, relationships among, and general symptomatology of WMV, ZYMV, PRSV-W and MWMV

Watermelon mosaic virus (WMV) causes major loss in yield and quality of cucurbit crops worldwide (Purcifull et al, 1984a). With an extensive range of natural hosts, WMV has been reported in Australia, Chile, France, Hungary, Iran, Israel, Italy, Japan, Mexico, New Zealand, and Venezuela. Reports of reduction in yield and quality in the United States due, in part, to WMV dates back to the late 1940's (Webb and Scott, 1965).

In 1965, Webb and Scott described WMV based on host range and serological properties. They divided the isolates studied into two groups. Isolates that did not infect non-cucurbitaceous plants were designated as WMV-1 and isolates that infect species of Leguminosae, Chenopodiaceae and Euphorbiaceae were designated WMV-2. WMV-1 and WMV-2 were first considered to be strains of the same virus until biological, serological and cDNA hybridization studies indicated WMV-1 was a strain of PRSV and reclassified as the watermelon strain of papaya ringspot virus (PRSV-W) (Gonsalves and Ishii, 1980; Purcifull et al, 1984a,b). Later, this finding was reinforced by coat protein sequence analysis (Quemada et al, 1990).

Symptoms of WMV generally consist of a systemic mosaic and/or mottling pattern of the foliage with occasional leaf distortion. A reduction in

the production and quality of fruit following WMV infection is observed in cucumber and other cucurbits (Purcifull et al, 1984a).

Zucchini yellow mosaic virus (ZYMV) was first observed in zucchini crops of northern Italy in 1973 (Lisa et al, 1981). ZYMV has since been detected in Asia, Australia, central Europe, France, Germany, Israel, Japan, Lebanon, Mauritius, Morocco, Spain and the United Kingdom (Lisa and Lecoq, 1984; Davis, 1986; Sammons et al, 1989). In the United States, Florida reported the occurrence of ZYMV in 1981, Connecticut, California and Oregon in 1982, New York in 1983, New Jersey and Arkansas in 1985 (Sammons et al, 1989) and the Hawaiian Islands in 1988 and 1989 (Ullman et al, 1991). The first report of ZYMV in Ontario occurred in 1989 (Stobbs and Van Schagen, 1989).

ZYMV is serologically distantly related to WMV, PRSV-W, bean yellow mosaic virus and to amaranthus leaf mottle virus (Lisa and Lecoq, 1984). Strains of ZYMV differ in symptomatology but symptoms include mosaic, yellowing, shoestringing, stunting and fruit distortion. Certain ZYMV strains induce symptoms similar to those of other major cucurbit viruses such as cucumber mosaic virus, PRSV-W and WMV and two or more of these viruses will frequently occur together.

As with WMV and ZYMV, the watermelon strain of papaya ringspot virus (PRSV-W) is considered a serious threat to cucurbit production and is widely distributed geographically including Australia, the Caribbean,

France, Germany, India, Italy, the middle east, South America and the United States (Purcifull et al, 1984b).

PRSV consists of three pathotypes - the papaya strain (PRSV-P), a pathogen of papaya and cucurbits, the watermelon strain (PRSV-W), a pathogen of cucurbits, but not papaya, and a strain isolated from squash (PRSV-T) which also does not infect papaya (Baker et al, 1991). Serologically, PRSV is closely related to WMV and ZYMV but distantly related to bean yellow mosaic virus, potato virus Y, and tobacco etch virus (Purcifull et al, 1984b). The type W strain of PRSV causes severe systemic mottling and/or dark green blistering of leaves. Distortion of fruit prevails following infection rendering them unmarketable.

The Moroccan watermelon mosaic virus (MWMV) was first reported causing severe damage to cucurbits in Morocco in 1974 (Fischer and Lockhart, 1974). Since then, isolates of this virus have been detected in Cameron, the Canary Islands, Niger, South Africa, Spain, and Zimbabwe (Van der Meer and Garnet, 1987; Quiot-Douine et al, 1990; McKern et al, 1993).

Based on biological properties, Fischer and Lockhart (1974) first classified MWMV as a strain of WMV (formerly WMV-2). However, subsequent comparative studies, based on biological and serological properties, indicated this virus was related to but distinct from PRSV-W (formerly WMV-1) (Quiot-Douine et al, 1990). To clarify the taxonomic

relationship of this virus, McKern, et al (1993) compared a Moroccan isolate to PRSV-W, WMV and soybean mosaic virus (a potyvirus related to WMV) using high performance liquid chromatography of tryptic peptides and limited amino acid sequencing of the coat protein. Their results concluded that the isolate from Morocco was a distinct potyvirus. Similarly, sequence analysis of cDNA clones of the coat protein gene show approximately 60% homology with other cucurbit potyviruses confirming that MWMV is a distinct potyvirus (Lanina and Grumet, unpublished).

Symptoms of MWMV consist of a foliar mosaic, green blistering, shoe stringing and general stunting of the plant. Fruits show distortion, knobby-like areas and color break (Fisher and Lockhart, 1974).

1.4 Sources of resistance in cucumber to WMV, ZYMV and PRSV-W

Naturally occurring resistances to viral disease have been identified in existing cultivars, primitive cultivars or landraces, closely related wild or cultivated species and genera of the same botanical family (Provvidenti, 1989). Provvidenti and Hampton (1992) report on sources of resistance to 56 viruses of the *Potyviridae* in 334 plant species. Of the *Cucurbitaceae*, genes for resistance to WMV, ZYMV, PRSV-W and MWMV have been identified in watermelon: *Citrullus colocynthis, C. ecirrhosus, C. lanatus;* squash and pumpkins: *Cucurbita ecuadorensis, C. ficifolia, C. foetidissima,*

C. maxima, C. moschata, C. pedatifolia; and melon and cucumbers: *Cucumis melo, C. sativus,* and *C. metuliferus.*

In cucumber (*Cucumis sativus* L.), Provvidenti (1985) identified two accessions that possessed resistance to the most common potyviruses that afflict cucurbits. Resistance to ZYMV, WMV, and PRSV-W was identified in a single plant selection from the Chinese cucumber cultivar 'Taichung Mou Gua' (TMG-1) and resistance to PRSV-W was identified in a cultivar from South America, 'Surinam Local'. The inheritance of resistance to ZYMV, PRSV-W and WMV in TMG-1 has been characterized. In TMG-1, resistance to ZYMV is conferred by a single recessive gene (Provvidenti, 1987) and resistance to PRSV-W by a single dominant gene (Wai and Grumet, 1995a). Inheritance of resistance to WMV in TMG-1 exhibits a tissue-specific expression involving two independent types of resistances (Wai and Grumet, 1995b). In addition, their research suggests the ZYMV resistance gene appears the same as, or tightly linked to, one of the WMV resistance genes. The inheritance of resistance to PRSV-W in the cucumber cultivar 'Surinam Local' was characterized by Wang, et al, in 1984. Their study revealed a single recessive gene in Surinam confers resistance to PRSV-W. An additional source of resistance to ZYMV was identified in a greenhouse grown Dutch hybrid 'Dina' with characterization of this resistance determined by Abul Hayja and Al-Shahwan (1991) as a single recessive gene.

1.5 Cucumber genome and linkage analysis

The Cucurbitaceae are among the more important plant families that supply the human population with edible products (Wehner and Robinson, 1991). The three different genera of the cucurbit family are *Cucumis* (cucumber and melons), *Citrullus* (watermelon) and *Cucurbita* (squash and pumpkins). *Cucumis sativus* L, the cucumber, is grown from the extremes of the temperate zones to the tropics. This species is believed to have originated in Asia with ancestry similar to *Cucumis sativus* var. hardwickii.

The genetics of the cucumber (2n=14) has been extensively studied with numerous morphological and disease resistance genes having been identified since the 1930's (Robinson et al, 1976). Since then, efforts have been made to analyze linkage distances for the construction of genetic maps through the use of RFLP, RAPD, isozyme, morphological and disease resistance markers.

In 1987, Fanourakis and Simon investigated the linkage relationship of 15 simply inherited traits to initiate construction of a genetic map of cucumber. Linkage analysis arranged 13 of the 15 loci into three linkage groups. In 1990, Pierce and Wehner, in their review of genetic linkages in cucumber, assembled a morphological linkage map, identifying 40 map positions on six linkage groups. Knerr and Staub in 1992, through joint segregation analyses of 14 polymorphic isozyme loci, assigned 12 loci to four

linkage groups. Most recently, in 1994, Kennard, et al, constructed a 58 point genetic map assigned to ten linkage groups.

As characterization of genetically important traits in cucumber continue to be identified and linkage distances analyzed, construction of a detailed, saturated map of the cucumber will be attained.

1.6 Response to viral infection in plants - Definition of terms

Definitions of terms which describe different interactions and responses of a host and a pathogen have evolved over the years and continue to change. This is in part due to disagreement between mycologists, virologists, and plant breeders as to the use of terms and in part due to not fully understanding the mechanisms involved in host-pathogen interactions (Fraser, 1986). In as much as this terminology is evolving, defining the terms that have been used to describe the different host-pathogen interactions and responses seem necessary. The following are definitions of terms used to describe host-pathogen interactions and responses obtained from several sources including Cooper and Jones, 1983; Fraser, 1986,1988.

Plants which allow virus multiplication and possible (but not always) visible disease symptoms are *susceptible* and considered *host* to that particular virus. *Multiplication* is defined as an increase of virus in time and space. If an individual plant, or plant population, does not allow multiplication of a particular virus and remains unaffected after repeated

inoculations, it is considered to possess *nonhost immunity*. Inhibition of virus multiplication or inhibition of pathogenic effect on a host is termed *resistance*. This resistance can operate at the cellular, plant or population level. Individual plants or varieties that possess resistance to a particular virus that normally affects that species of plant are considered to possess *constitutive resistance*, or at time referred to as *cultivar resistance*. *Tolerance* is a subjective description of disease severity and can present itself as a complete absence, or only a mild degree, of symptoms causing little or no loss in vigor or yield. *Hypersensitivity* is considered a type of pathological response which takes the form of localized lesions or necrosis associated with restricted virus invasion. *Passive resistance* has been used to describe individuals that do not readily become infected, but once infected, replication of the disease can occur. Plants can possess resistance to a virus but be sensitive. *Sensitivity* is a subjective description of disease severity associated with conspicuous symptoms that may diminish the rate or amount of plant growth or yield.

A virus that multiplies and successfully infects a host is *pathogenic* to that host. A virus that fails to establish infection is *non-pathogenic* to nonhost plant species. *Virulence* is defined as the ability of a particular strain of virus to multiply or cause disease within individuals of host species that posses genes for resistance. Virus isolates not capable of multiplication

and disease within plant species containing susceptible individuals are *avirulent*.

1.7 Classical genetic analysis of plant resistance to viruses

Classical genetic analysis of resistance to viruses in plants has been accomplished by evaluating the progeny of crosses between true-breeding resistant and susceptible lines to inoculation with a particular virus (Fraser, 1986). To evaluate the type of genetic control for resistance upon inoculation, the observed genetic segregation ratios are compared to the expected genetic segregation ratios. Scoring for response or reaction in plants to virus inoculation is generally based on visual assessment of symptom severity on individual plants, percentage of plants showing disease symptoms, back inoculation to an indicator host, and quantitative measurement of virus multiplication.

Resistance to viruses in most plant species is simply inherited monogenetically (Fraser 1986; Provvidenti and Hampton, 1992). This single locus resistance can manifest itself as either dominant or recessive and may provide resistance to one particular virus, be pathotype specific, confer resistance to two or more distinct viruses, or be tightly linked with other resistance gene(s), in clusters, conferring resistance to multiple viruses or strains.

Many simply inherited monogenetic disease resistances have been identified in literature (Provvidenti and Hampton, 1992). For example, resistance to blackeye cowpea mosaic virus in *Vigna unguiculata* is governed by a single dominant gene (Ouattara and Chambliss, 1991) and resistance to pea seed-borne mosaic virus in *Pisum sativum* is conferred by a single recessive gene (Hagedorn and Gritton, 1973). Examples of strain or pathotype specific resistance includes a single dominant gene in *Phaseolus vulgaris* that confers resistance to the PV2 strain of bean yellow mosaic virus but susceptibility to other strains (Schroeder and Provvidenti, 1968) and a single recessive gene in *Pisum sativum* that confers resistance to a third pathotype of pea seed-borne mosaic virus inherited independently but possibly linked with two other single recessive genes conferring resistance to other pathotypes of pea seed-borne mosaic virus (Provvidenti and Alconero, 1988).

The existence of simply inherited genes, or clusters of separate tightly-linked genes, that confer resistance to two or more distinct potyviruses have been described previously in several species. For example, in *Cucurbita moschata*, a single dominant gene confers resistance to both ZYMV and WMV (Gilbert-Albertini et al, 1993), in *Solanum stoloniferum,* a single dominant gene confers resistance to potato virus A and potato virus Y (Cockerham, 1970), and resistance to WMV and bean yellow mosaic virus is conferred by a single recessive gene in *Pisum sativum* (Schroeder and Provvidenti, 1971).

In *Phaseolus vulgaris*, the possibility of a single gene, or cluster of tightly linked genes cosegregating as a unit with the *I* gene, condition resistance and/or lethal necrosis to a set of nine potyviruses including bean common mosaic virus, WMV, blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, azuki bean mosaic virus, Thailand Passiflora virus, soybean mosaic virus, passionfruit woodiness virus-K and ZYMV. (Kyle and Dickson, 1988; Fisher and Kyle, 1994). In *Pisum sativum*, well defined clusters of tightly-linked loci conferring resistance to a total of 11 potyviruses are located on two chromosomes (Provvidenti and Hampton, 1993; Provvidenti and Niblett, 1994). The first cluster, on chromosome 2, contains resistance to WMV, bean yellow mosaic virus, pea mosaic virus, the NL-8 strain of bean common mosaic virus, the lentil strain of pea seed-borne mosaic virus, clover yellow vein virus, and the Australian strain of passionfruit woodiness virus. The second cluster, on chromosome 6, includes resistance to three pathotypes of pea seed-borne mosaic virus, clover yellow vein virus, and white lupin mosaic virus.

Clusters of tightly linked genes have also been identified for disease resistance other than viral diseases. Disease resistance genes in *Lactuca* L. spp. have been arranged in three distinct clusters (Witsenboer et al, 1995). The largest cluster contains eight genes (*Dm1, Dm2, Dm3, Dm6, Dm13, Dm14, Dm15, Dm16*) for resistance to downy mildew plus a gene (*Ra*) for resistance to root-aphids (Crute and Dunn, 1980; Farrara et al, 1987). The

second largest cluster contains two downy mildew genes (*Dm5/8, Dm10*), a dominant gene (*Tu*) conferring resistance to turnip mosaic virus and a recessive gene (*plr*) conferring resistance to root downy mildew (Vandemark et al, 1992; Zink and Duffus, 1973). Three additional downy mildew resistance genes (*Dm4, DM7, Dm11*) comprise the third cluster (Hulbert and Michelmore, 1985).

Not all disease control systems in plants are simple. Single locus resistance can also manifest itself as incomplete dominance or gene dosage-dependent. Judgment as to whether a single locus resistance is dominant or recessive is typically based on evaluating the F_1 and segregating progeny of the cross between pure-breeding resistant and susceptible parental lines for symptoms of virus infection post inoculation with a particular virus. This subjective observation of phenotypic symptom expression can be misleading. An example of this is found in the resistance to tobacco mosaic virus in tomato (Fraser and Loughlin, 1980). In the heterozygous state, the resistance allele appears completely dominant with no viral symptoms observed. However, measurement of virus multiplication of the heterozygote revealed virus replication which is suggestive of a gene dosage-dependent resistance. Resistance to soybean mosaic virus in common bean is reported to be governed by an incompletely dominant gene (Kyle and Provvidenti, 1993). In the homozygous state, no detectable systemic symptoms were observed, however, virus could be detected in inoculated leaves. In the

heterozygous state, under field or summer greenhouse conditions, individual plants develop local chlorosis with systemic symptoms consisting of a mild to moderate mottling. Plants, however, remain vigorous or only slightly stunted. Interestingly, the incomplete dominant resistance allele of soybean mosaic virus will appear completely dominant when plants are inoculated and grown under winter greenhouse conditions. An additional example of a single incomplete dominant system of control is reported in sugarbeet which possesses resistance to beet mosaic virus (Lewellen, 1973). Evaluation of the parental and segregating populations, at an early stage of infection (10-14 days post virus inoculation) fell into discrete 3:1 [resistant(R):susceptible(S)] classes for the F_2 progeny and 1:0 (R:S) for the progeny backcrossed to the resistant parent. However, at a later stage of infection (20-30 days post virus inoculation) and depending on the time of year and greenhouse temperatures, the F_2 progeny segregated as a 1:2:1 (R:intermediate:S) ratio and the backcross to the resistant parent segregated as a 1:1 (R:intermediate) ratio.

Effectiveness of resistance may depend on the genetic background of the host cultivar. An example of this is found in barley which possesses tolerance to barley yellow dwarf virus (Jones and Catherall, 1970). Reduced effectiveness of tolerance, conferred by an incompletely dominant gene (Yd_2) was observed with slow growth rate considered either the result of host genetic factors or environmental conditions. Interestingly, the effectiveness

of tolerance was recovered when introduced into rapidly growing genotypes. In a follow-up study, Catherall, et al (1970), evaluated five gene donor varieties that showed different levels of tolerance to barley yellow dwarf virus. The one variety showing the greatest tolerance was the fastest growing while the least tolerant was the slowest growing variety. When the tolerance factors from these varieties were introduced into similar genetic backgrounds, the tolerance factor from the fastest growing variety consistently provided the highest tolerance. Although the existence of modifying genes effecting the expression of the tolerance gene could not be ruled out, these findings were suggestive of a series of alleles occurring at the Yd_2 gene locus differing in their effectiveness and their dominance relationships.

The inheritance of resistance in plants to viral disease also can involve more complex systems of control. This complexity can involve two or more resistance factors, operating independently or additively. Kyle and Provvidenti (1987) report on two independent dominant genes that confer resistance to WMV in *Phaseolus vulgaris* L. This pair of independent genes, *Wmv* and *Hsw*, are not pathotype-specific but can be distinguished phenotypically at both low and high temperatures. The inheritance of resistance to tobacco etch virus in *Nicotiana tabacum* cv. Havana 307 was investigated by Rufty, et al (1988). In their investigation, parent and progeny segregation ratios did not fit a simple Mendelian gene model but one

of resistance due to a few genes (possibly as few as two) with additive effects based on a high frequency of resistant genotypes observed in the F_2 generation, and the results of generation means analysis. Goodrick, et al, in 1991, reported on the inheritance of resistance to cowpea chlorotic mottle virus in soybean. Their findings did not support a monogenetic control of resistance but one of two recessive genes that conditioned resistance. A final example of a more complex system of control is found in resistance to wheat streak mosaic virus in maize (McMullen and Louie, 1991; McMullen et al, 1994). McMullen and Louie (1991) investigated the inheritance of resistance to wheat streak mosaic virus in maize and identified a single dominant gene for resistance (*Ws1*). However, in analyzing the segregating ratios of the parental lines and progeny, additional genetic factors seemed involved as different symptomatic responses were observed. To identify the additional genetic factor(s) controlling resistance to wheat streak mosaic virus, McMullen, et al (1994), through the use of bulked segregant analysis were able to identify two additional genes. In addition, through the use of RFLP analysis, they were able to determine the genotypes of the different symptomatic responses to wheat streak mosaic virus infection. Plants that exhibited a susceptible response of general mosaic were determined to be *wsm1/wsm1, wsm2/wsm2* and *wsm3/wsm3* while the genotype of symptom-free individuals were *Wsm1/--, Wsm2/--* and *Wsm3/--*. Of interest were the

individual plants that exhibited dispersed chlorotic spots and rings. This genotype was determined to be *wsm1/wsm1, wsm2/wsm2* and *Wsm3/--*.

1.8 Purpose of thesis

Potyviruses, especially ZYMV, WMV, and PRSV-W, cause severe losses in cucumber and other cucurbit crops worldwide (Lisa and Lecoq, 1984; Purcifull et al, 1984a,b). Recently, an additional virus infecting cucurbits, MWMV, has been identified to be a distinct member of the potyviruses (McKern et al, 1993).

Potyvirus resistance can be governed by a single gene conferring resistance to one specific pathotype, one particular virus, several distinct viruses, or be tightly linked to other resistance gene(s) conferring resistance to multiple viruses or strains (Fraser, 1986; Provvidenti and Hampton, 1992). Disease resistance may also involve a more complex system such as incomplete dominance (or gene dosage-dependence), effectiveness of resistance may depend on the genetic background of the host, or may involve two or more resistance factors, operating independently or additively.

A number of genes governing resistance to potyviruses in cucumber have been identified and characterized. Inbred lines derived from 'TMG' govern resistance to ZYMV, WMV, PRSV-W (Provvidenti, 1985) and more recently ZYFV (Gilbert-Albertini et al, 1995). Abul Hayja and Al-Shahwan

(1991) described resistance to ZYMV in the Dutch hybrid 'Dina' and Wang, et al (1984), reported resistance to PRSV-W in the cultivar 'Surinam Local'.

In TMG, resistance to ZYMV is conferred by a single recessive gene (Provvidenti, 1987), resistance to PRSV-W by a single dominant gene (Wai and Grumet, 1995a), and resistance to ZYFV by a single recessive gene (Gilbert-Albertini et al, 1995). Resistance to WMV in TMG exhibits a tissue-specific expression involving two independent genes (Wai and Grumet, 1995b). In addition, resistance to WMV and resistance to ZYMV in TMG appear to be due to the same, or tightly linked genes (Wai and Grumet, 1995b). In Surinam Local, resistance to PRSV-W is conferred by a single recessive gene (Wang et al, 1984) and in the Dutch hybrid Dina, resistance to ZYMV is conferred by a single recessive gene (Abul Hayja and Al-Shahwan, 1991).

The purpose of this study is (1) determine if TMG possesses resistance to MWMV and, if so, to investigate the genetic relationship, if any, between resistance to MWMV and the resistance to other cucurbit potyviruses, (2) determine the genetic relationship between resistance alleles in TMG to other sources of resistance in cucumber to potyviruses and (3) determine the relationship of different potyviruses to each other.

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CHAPTER 2

Inheritance of resistance to the Moroccan watermelon mosaic virus in the cucumber line TMG-1 and cosegregation with zucchini yellow mosaic virus resistance

ABSTRACT

Inbred lines derived from the Chinese cucumber cultivar, 'Taichung Mou Gua' (TMG), have been identified to be resistant to several potyviruses including: zucchini yellow mosaic virus (ZYMV), zucchini yellow fleck virus (ZYFV), watermelon mosaic virus (WMV) and the watermelon strain of papaya ringspot virus (PRSV-W). Recently, an additional virus that infects cucurbits, the Moroccan watermelon mosaic virus (MWMV), has been identified to be a distinct member of the potyvirus group. This study demonstrates that TMG-1 possesses resistance to MWMV. Rub or aphid inoculated TMG-1 seedlings remain free of symptoms. Progeny analyses of the F_1, F_2 and backcross generations show that resistance to MWMV is conferred by a single recessive gene. Sequential inoculation of progeny possessing resistance to ZYMV followed by MWMV (or MWMV followed by ZYMV) and analysis of F_3 families derived from F_2 individuals selected for resistance to ZYMV indicate that both resistances are conferred by the same gene, or two tightly linked genes.

INTRODUCTION

Cucumbers (*Cucumis sativus* L.) and other cucurbit crops are subject to severe losses due to an array of potyviruses including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and the watermelon strain of papaya ringspot virus (PRSV-W) (Lisa and Lecoq, 1984; Purcifull et al, 1984a,b). An additional potyvirus, the Moroccan watermelon mosaic virus (MWMV), was first reported causing severe damage to cucurbits in Morocco in 1974 (Fischer and Lockhart, 1974). Since then, isolates of this virus have been detected in Cameron, the Canary Islands, Niger, South Africa, Spain and Zimbabwe (Van der Meer and Garnet, 1987; Quiot-Douine et al, 1990; McKern et al, 1993). Although the relationship of MWMV to other potyviruses had not been well defined, recent amino acid and nucleic acid sequence analysis of the coat protein and its cDNA has verified that MWMV is a distinct member of the potyvirus group (McKern et al, 1993; Lanina and Grumet, unpublished). Predicted coat protein amino acid sequences indicate that the core portion of the MWMV coat protein shares approximately 73% identity with PRSV and approximately 67% sequence identity with WMV and ZYMV, respectively (Lanina and Grumet, unpublished).

Sources of resistance to MWMV have been identified in *Cucumis metuliferus, Citrullus ecirrhosus, Coccinia sessifolia* and *Luffa aegyptica* (Provvidenti and Hampton, 1992) but not for *Cucumis sativus*. However, a number of genes governing resistance to various other potyviruses in

cucumber have been identified. One particularly interesting genotype, the Chinese cucumber cultivar 'Taichung Mou Gau' (TMG), is resistant to at least four different potyviruses. Inbred lines derived from TMG have been found to possess resistance to ZYMV, WMV, PRSV-W (Provvidenti, 1985), and zucchini yellow fleck virus (ZYFV) (Gilbert-Albertini et al, 1995).

The potyvirus resistances in TMG show various genetic controls. Resistance to ZYMV is conferred by a single recessive gene (Provvidenti 1987a) as is resistance to ZYFV (Gilbert-Albertini et al, 1995); resistance to PRSV-W is due to a single dominant, or incompletely dominant, gene (Wai and Grumet, 1995a). Resistance to WMV in TMG involves two types of resistances (Wai and Grumet, 1995b). One is expressed in the cotyledons and throughout the plant, the second is expressed only in the true leaves. Interestingly, the gene conferring resistance to ZYMV appears to be the same as, or tightly linked to, one of the WMV resistance genes.

Since the cucumber cultivar TMG possesses resistance to several potyviruses, we sought to determine if it also possessed resistance to MWMV and, if so, characterize the inheritance of resistance. In addition, as previous studies with TMG indicated that the resistances to ZYMV and WMV may be controlled by the same, or tightly linked, genes (Wai and Grumet, 1995b), we also sought to determine if there is a genetic relationship between resistance to ZYMV and MWMV.

MATERIALS AND METHODS

Cucumber genotypes

The inbred cucumber (*Cucumis sativus* L.) line 'TMG-1', resistant to ZYMV, WMV, and PRSV-W (Provvidenti, 1985) was provided by Dr. Jack Staub (USDA, University of Wisconsin at Madison). The two susceptible parental genotypes used in this investigation were the inbred lines 'Wisconsin 2757' (WI-2757; Peterson et al, 1982; provided by Dr. Jack Staub) and 'Straight-8' (ST-8; W. Atlee Burpee & Company, Warminster, PA).

Production of the F_1, F_2 and backcross progeny of WI-2757 and TMG-1 was described by Wai and Grumet (1995a). F_3 families, used to analyze the relationship between resistance to ZYMV and MWMV, were produced by first screening 1,479 F_2 (WI-2757 x TMG-1) individuals for resistance to ZYMV in the greenhouse. Three-hundred-seventy-three ZYMV resistant F_2 individuals [3:1 (susceptible:resistant); $X^2 = 0.08$; non-significant] were then transplanted to the field. As WI-2757 is gynoecious (dominant trait; Peterson et al, 1982), monoecious individuals were identified and self-pollinated by hand under controlled conditions. Fifty-one F_3 families were produced. The progeny of the cross between ST-8 and TMG-1 were produced in the greenhouse. The reciprocal F_1 progeny were self-pollinated to produce the F_2 generation. Backcrosses were made to both parents using the F_1 (ST-8 x TMG-1) genotype.

Virus inocula and inoculation procedures

MWMV (provided by Dr. Purcifull, University of Florida, Gainesville, FL) and ZYMV (Connecticut strain) were propagated in *Cucurbita pepo* L. cvs. 'Black Beauty' (SeedWay Inc., Elizabethtown, PA) or 'Midas' (Willhite Seed, Poolville, TX) and maintained in a growth chamber (16-h day, 24°C constant temperature, ca 300 umol photons M^{-2} s^{-1}). Purity of the virus source was verified by ELISA, as described by Wai and Grumet (1995a), and by the use of the differential host, *Phaseolus vulgaris* cv. 'Black Turtle 2' (Provvidenti, 1983).

Young symptomatic leaves were harvested two to four weeks post inoculation and macerated in ice-cold 20mM sodium phosphate buffer, pH 7.0, in a pre-chilled mortar and pestle. Cotyledons of 7-10 day old seedlings, or the upper surface of the fully expanded second and half expanded third true leaves, were lightly dusted with 320-grit Carborundum (Fisher Scientific, Pittsburgh, PA) and rub inoculated with virus-infected sap (approximately 1:4 dilution leaf material:buffer) using sponge plugs. Mock-inoculated control plants were included in each experiment. All non-biological materials were sterilized prior to use.

TMG-1, WI-2757 and ST-8 were also inoculated with MWMV using aphids. Virus-free aphids were harvested from *Nicotiana tabacum* cv. 'Burley' stock plants and allowed to feed on symptomatic virus-infected leaves of *Cucurbita pepo* L. for one to three minutes. The aphids were

transferred to young cucumber seedlings (ten/plant) and allowed to feed for two hours. All aphids were then removed by hand. Aphid inoculated cucumber seedlings were transferred to a controlled growth chamber environment, sprayed with the insecticide Orthene (Monsanto, St. Louis, MO), and observed for symptom development.

Experimental designs and data analysis

Seed for all experiments were pregerminated in a 30°C incubator for 24 hours then sown in the greenhouse into 10.2 cm clay pots filled with Baccto growing medium (Professional Planting Mix, Michigan Peat Company, Houston, TX). Upon emergence of the cotyledons, a fertilization regimen of 300 ppm N-P-K (Peters Professional All-Purpose Fertilizer, 20-20-20, Grace-Sierra Horticultural Products, Milpitas, CA) was applied three times per week. Greenhouse temperatures ranged from 25°C to 35°C throughout the year.

The F_2 and backcross populations were inoculated at the cotyledon stage. Sixteen rows of ten plants/row were interspersed with five internal control rows consisting of inoculated, mock-inoculated and non-inoculated parental and F_1 progeny. Similarly, tests of F_3 families included ten individuals per family with five control rows evenly distributed along the bench. Sequential inoculation experiments consisted of cotyledon inoculation of parental genotypes and their progeny with one virus followed by true leaf inoculation of the resistant individuals with the second virus. Sequential

inoculation experiments were performed in both directions: cotyledon inoculation with MWMV followed by true leaf inoculation with ZYMV or cotyledon inoculation with ZYMV followed by true leaf inoculation with MWMV. Additional inoculated, mock-inoculated and non-inoculated control plants were included for the true leaf inoculation portion of the experiment to confirm successful virus inoculation. All experiments included border rows of ST-8 (susceptible genotype) as a further check for any possible variation in the inoculation technique and/or expression of viral symptoms.

In all experiments, plants were visually scored as either resistant (symptom-free) or susceptible (evidence of virus) when symptom development was optimal (approximately seven days post inoculation for ZYMV and approximately 14 days post inoculation for MWMV). Segregation ratios were analyzed by Chi square analysis.

RESULTS

Response of TMG-1 to inoculation with MWMV

Symptoms of MWMV generally developed 14 days post cotyledon or true leaf inoculation. Following rub inoculation with MWMV, TMG-1 remained symptom-free. Symptom expression on the susceptible genotypes, WI-2757 and ST-8, showed a mild systemic rugosity and silvering of the true leaves. Symptom intensity varied during the course of plant growth, the rugosity and silvering of the true leaves would fade occasionally but then

resurge to full expression. Since potyviruses are normally transmitted by aphids in nature, the response of TMG-1 to MWMV also was tested using aphid inoculation. As for rub inoculation, TMG-1 remained symptom-free while WI-2757 and ST-8 developed mild systemic rugosity and silvering of the true leaves (data not shown).

Inheritance of resistance to MWMV in TMG-1

The F_1 progeny of the crosses between ST-8 and TMG-1 and WI-2757 and TMG-1 reacted in a similar fashion and intensity as the susceptible parents, showing systemic mild rugosity and silvering of the true leaves (Table 2.1). The F_2 progeny (ST-8 x TMG-1, TMG-1 x ST-8 or WI-2757 x TMG-1) segregated in a 1:3 [resistant (R):susceptible (S)] ratio while the backcrosses to the resistant TMG-1 parent segregated in a 1:1 (R:S) ratio. Progeny of the backcrosses to the susceptible parents were all susceptible. These segregation ratios support a model in which resistance to MWMV in TMG-1 is conferred by a single recessive gene (proposed gene designation *mwmv*).

Previous studies by Wai and Grumet (1995b) indicated that resistance to WMV in TMG-1 involves two types of resistances, one of which is expressed only in the true leaves. To determine if TMG-1 had a similar true leaf-specific system of resistance to MWMV, the parental genotypes of TMG-1 and ST-8 and their progeny were inoculated with MWMV at the true leaf

Table 2.1 Segregation data for response to cotyledon inoculation with MWMV in populations derived from the inbred cucumber lines TMG-1, WI-2757, and Straight-8.

Parent or progeny	Number of plants		Expected ratio (R:S)[a]	X^2
	Resistant	Susceptible		
TMG-1 (T)	36	0		
Straight-8 (ST8)	0	36		
WI-2757 (2757)	0	12		
F1 (ST8xT) (TxST8)	0	48	0:1	
F1 (2757xT)	0	12	0:1	
F2 (ST8xT)[b] (TxST8)	78	241	1:3	0.037 ns
F2 (2757xT)	39	121	1:3	0.008 ns
BC (ST8xT) xT[c] Tx (ST8xT)	111	129	1:1	1.204 ns
BC (2757xT) xT	73	67	1:1	0.178 ns
BC (ST8xT) x ST8 ST8 x (ST8xT)	0	160	0:1	
BC 2757 x (2757xT)	0	20	0:1	

ns, non-significant X^2 value

[a]expected ratios based on a single recessive gene model, R = resistant, S = susceptible.

[b]data pooled from two independent experiments. Each experiment fits the predicted ratios X^2exp1 = 0.000, X^2exp2 = 0.076; X^2 homogeneity = 0.053, df = 1.

[c]data pooled from two independent experiments. Each experiment fits the predicted ratios X^2exp1 = 1.406, X^2exp2 = 0.012; X^2 homogeneity = 0.32, df = 1.

stage (Table 2.2). Symptom development following true leaf inoculation occurred approximately 14 days post inoculation. Segregation ratios revealed no difference between cotyledon and true leaf inoculation with MWMV.

Genetic relationship between resistance to MWMV and resistance to ZYMV in TMG-1

To investigate whether a relationship exists between the resistance to MWMV and resistance to ZYMV in TMG-1, two approaches were taken. The first approach was sequential inoculation. This procedure consisted of cotyledon inoculation of TMG-1, ST-8 and their progeny with MWMV followed by true leaf inoculation of the resistant individuals with ZYMV (Table 2.3A). In a separate experiment, this procedure was reversed inoculating the cotyledons with ZYMV followed by true leaf inoculation of the resistant individuals with MWMV (Table 2.3B). In all experiments, additional control plants were included to verify successful inoculation at the true leaf stage. Symptoms of MWMV in the susceptible genotypes, WI-2757 and ST-8, were as described earlier. Symptoms of ZYMV, a systemic mosaic pattern of the true leaves, generally developed on the susceptible genotypes (WI-2757 and ST-8) seven days post cotyledon or true leaf inoculation. The resistant genotype, TMG-1, remained free of symptoms for both MWMV and ZYMV rub inoculation. In all cases, those individuals resistant to cotyledon inoculation with the first virus remained symptom-free upon true leaf inoculation with the second virus.

Table 2.2 Segregation data for response to true leaf inoculation with MWMV in populations derived from the inbred cucumber lines TMG-1 and Straight-8.

Parent or progeny	Number of plants		Expected ratio (R:S)[a]	X^2
	Resistant	Susceptible		
TMG-1 (T)	16	0		
Straight-8 (ST8)	0	16		
F1 (ST8xT) (TxST8)	0	34	0:1	
F2 (ST8xT) (TxST8)	43	109	1:3	0.711 ns
BC (ST8xT) x T[b] T x (ST8xT)	76	83	1:1	0.226 ns
BC (ST8xT) x ST8 ST8 x (ST8xT)	0	80	0:1	

ns, non-significant X^2 value
[a]expected ratios based on a single recessive gene model, R = resistant, S = susceptible.
[b]data pooled from two independent experiments. Each experiment fits the predicted ratios X^2exp1 = 0.81, X^2exp2 = 0.012; X^2 homogeneity = 1.01, df = 1.

Table 2.3 **Response to sequential inoculation with ZYMV and MWMV in populations derived from the inbred cucumber lines TMG-1 and Straight-8.**

A. Cotyledon inoculation with MWMV followed by true leaf inoculation of resistant individuals with ZYMV.

Parent or progeny	Total plants	Cotyledon inoculation with MWMV — Number of plants		Sequential inoculation of MWMV resistant individuals with ZYMV at true leaf stage — Number of plants	
		Resistant	Susceptible	Resistant	Susceptible
TMG-1 (T)	18	18	0	18	0
Straight-8 (ST8)[a]	9	0	9	–	–
Straight-8 (ST8)[b]	9	–	–	0	9
F1(ST8xT)[a]	18	0	18	–	–
F1(ST8xT)[b]	18	–	–	0	18
F2 (ST8xT)[c]	319	73	243	73	0
BC (ST8xT)xT[d]	160	71	89	71	0

[a]control plants to verify successful inoculation at cotyledon stage with MWMV
[b]control plants to verify successful inoculation at true leaf stage with ZYMV
[c]data pooled from two independent experiments. Each experiment fits the expected and predicted segregation ratios based on resistance to MWMV conferred by a single recessive gene. X^2exp1 = 0.68, X^2exp2 = 0.08; X^2 homogeneity = 0.16, df = 1
[d]data fits the expected and predicted segregation ratio based on resistance to MWMV conferred by a single recessive gene. $X^2 = 1.8$

B. Cotyledon inoculation with ZYMV followed by true leaf inoculation of resistant individuals with MWMV.

Parent or progeny	Total plants	Cotyledon inoculation with ZYMV — Number of plants		Sequential inoculation of ZYMV resistant individuals with MWMV at true leaf stage — Number of plants	
		Resistant	Susceptible	Resistant	Susceptible
TMG-1 (T)	20	20	0	20	0
Straight-8 (ST8)[a]	10	0	10	–	–
Straight-8 (ST8)[b]	10	–	–	0	10
F1(ST8xT)[a]	10	0	10	–	–
F1(ST8xT)[b]	10	–	–	0	10
F2 (ST8xT)[c]	160	40	120	40	0
BC (ST8xT)xT[d]	80	36	44	36	0

[a]control plants to verify successful inoculation at cotyledon stage with ZYMV
[b]control plants to verify successful inoculation at true leaf stage with MWMV
[c]data fits the expected and predicted segregation ratio based on resistance to ZYMV conferred by a single recessive gene. $X^2 = 0.00$
[d]data fits the expected and predicted segregation ratio based on resistance to ZYMV conferred by a single recessive gene. $X^2 = 0.62$

A second approach to evaluate whether a relationship exists between the resistance to MWMV and resistance to ZYMV was to screen F_3 families that were produced by self-pollinations of F_2 individuals selected for resistance to ZYMV. Progeny from each ZYMV resistant F_2 individual were inoculated either with MWMV or ZYMV (Table 2.4). As expected, all families were resistant to ZYMV. If the two resistances had been segregating independently, 9/16th of the F_3 families should be either susceptible or segregating for susceptibility to MWMV. There was, however, no segregation for susceptibility to MWMV either within or among the F_3 families screened.

DISCUSSION

To our knowledge, this is the first report of resistance to MWMV in *Cucumis sativus*. In this study, we have determined that the cucumber line TMG-1, which possesses resistance to ZYMV, WMV, and PRSV-W, also possesses resistance to MWMV. Rub and aphid inoculation with MWMV revealed TMG-1 to remain symptom-free while the susceptible genotypes, WI-2757 and ST-8, developed a mild systemic rugosity and silvering of the true leaves. Progeny analysis of the F_1, F_2, and backcross generations show that resistance to MWMV is conferred by a single recessive gene (*mwmv*). Sequential inoculation of progeny possessing resistance to ZYMV followed by MWMV (or MWMV followed by ZYMV) and analysis of F_3 families derived from F_2 individuals selected for resistance to ZYMV, indicate that both

Table 2.4 MWMV and ZYMV inoculation of F_3 families homozygous recessive at the *zym* locus derived from the inbred cucumber lines TMG-1 and WI-2757. Families were produced by self-pollinating F_2 individuals selected for resistance to ZYMV.

Potyvirus	No. F3 families[a] showing resistance	No. F3 families susceptible or segregating for resistance
MWMV	51	0
ZYMV	51	0

[a]tests of F_3 families included ten individuals/family.

resistances are conferred by the same gene, or two tightly linked genes (less than 1 cM; product-ratio method). Earlier work with TMG-1 indicated that the recessive gene for ZYMV resistance also conferred resistance to WMV (Wai and Grumet, 1995b). Since our current investigation suggests that the gene for ZYMV also confers resistance to MWMV, it appears a single recessive gene, or cluster of recessive genes, in TMG-1 governs resistance to at least three potyviruses, ZYMV, WMV and MWMV.

The existence of simply inherited genes, or clusters of separate tightly-linked genes, that confer resistance to two or more distinct potyviruses have been described previously in several species. For example, in *Cucurbita moschata*, a single dominant gene confers resistance to both ZYMV and WMV (Gilbert-Albertini et al, 1993), in *Solanum stoloniferum,* a single dominant gene confers resistance to potato virus A and potato virus Y (Cockerham, 1970), and resistance to WMV and bean yellow mosaic virus is conferred by a single recessive gene in *Pisum sativum* (Schroeder and Provvidenti, 1971). In *Phaseolus vulgaris*, the possibility of a single gene, or cluster of tightly linked genes cosegregating as a unit with the *I* gene, condition resistance and/or lethal necrosis to a set of nine potyviruses including bean common mosaic virus, WMV, blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, azuki bean mosaic virus, Thailand Passiflora virus, soybean mosaic virus, passionfruit woodiness virus-K and ZYMV. (Provvidenti et al, 1983; Kyle and Dickson, 1988; Fisher and Kyle, 1994). In *Pisum sativum*,

well defined clusters of tightly-linked loci conferring resistance to a total of 11 potyviruses are located on two chromosomes (Provvidenti and Hampton, 1993; Provvidenti and Niblett, 1994). The first cluster, on chromosome 2, contains resistance to WMV, bean yellow mosaic virus, pea mosaic virus, the NL-8 strain of bean common mosaic virus, the lentil strain of pea seed-borne mosaic virus, clover yellow vein virus, and the Australian strain of passionfruit woodiness virus. The second cluster, on chromosome 6, includes resistance to three pathotypes of pea seed-borne mosaic virus, clover yellow vein virus, and white lupin mosaic virus.

It has been postulated that single genes, or clusters of related genes, with conserved functions in plants could interrupt common pathogenic processes shared by closely related viruses (Kyle and Provvidenti, 1993; Fisher and Kyle, 1994). Sequence analyses have indicated that distinct potyviruses share 38-71% sequence identity in their coat protein, whereas strains within a given virus share 90-99% sequence identity (Shukla et al, 1994). It also has been suggested that viruses sharing intermediate levels of homology (74-88% sequence identity) can be grouped together to form subgroups within the potyviruses (Shukla et al, 1994). Of the viruses for which the *zym* gene or gene cluster in cucumber confers resistance, ZYMV and WMV share approximately 80% sequence and fall within the same subgroup (Fang and Grumet, 1990; Shukla et al, 1994). MWMV, however, does not appear to be a member of this subgroup as it shares only

approximately 60% sequence identity (Lanina and Grumet, unpublished). In cucumber, it is therefore possible that one gene can confer resistance across potyvirus subgroups or, alternatively, separate tightly-linked genes may be responsible for the multiple potyvirus resistance.

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CHAPTER 3

Multiple alleles for potyvirus resistance at the *zym* locus in cucumber

ABSTRACT

Sources of resistance to several potyviruses have been identified and characterized within the cucumber (*Cucumis sativus* L.) germplasm. Resistance to zucchini yellow mosaic virus (ZYMV) is found in the Dutch hybrid 'Dina' and in an inbred line derived from the Chinese cultivar 'Taichung Mou Gua' (TMG-1). Tests of allelism indicate that the genes for resistance to ZYMV in TMG-1 and Dina are at the same locus. However, they exhibit different phenotypes in response to cotyledon inoculation with ZYMV. Dina exhibits a distinct veinal chlorosis and accumulation of virus limited to the first and/or second true leaves. TMG-1, on the other hand, is symptom-free and lacks virus accumulation. In addition, the distinct veinal chlorosis phenotype in Dina is dominant to the symptom-free phenotype in TMG-1 and not due to a separate gene. These results indicate that a series of alleles occurs at the *zym* locus and that the alleles differ in their effectiveness and dominant relationships with $Zym^{+} > zym^{Dina} > zym^{TMG\text{-}1}$. In addition to ZYMV resistance, TMG-1 also possesses resistance to watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W) and

the Moroccan watermelon mosaic virus (MWMV), all of which appear to be at the same, or tightly linked loci. Our studies reveal that in addition to ZYMV resistance, Dina also possesses resistance to PRSV-W and MWMV, and that the gene for MWMV resistance is at the same locus as the ZYMV resistance. In addition, tests of allelism indicate that the gene for resistance to MWMV in TMG-1 and Dina are at the same locus. Interestingly, in contrast to ZYMV inoculation, Dina does not exhibit the distinct veinal chlorosis phenotype when inoculated with PRSV-W or MWMV. Collectively, these observations suggest that the genes conferring resistance to ZYMV, WMV, and MWMV may be part of a gene cluster for potyvirus resistance in cucumber.

INTRODUCTION

Cucumbers (*Cucumis sativus* L.) are subject to severe losses due to an array of potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W), zucchini yellow fleck virus (ZYFV), and the Moroccan watermelon mosaic virus (MWMV) (Lisa and Lecoq, 1984; Purcifull et al, 1984a,b; McKern et al, 1993; Gilbert-Albertini et al, 1995). Sources of resistance to several of these viruses have been identified and characterized within the cucumber germplasm. Resistance to ZYMV is found in the Dutch hybrid 'Dina' (Abul Hayja and Al-Shahwan, 1991) and in an inbred line

derived from the Chinese cultivar 'Taichung Mou Gua' (TMG-1) (Provvidenti, 1985). In addition, TMG also possesses resistance to WMV, ZYFV, PRSV-W and MWMV (Provvidenti, 1985; Gilbert-Albertini et al, 1995; Kabelka and Grumet, 1996).

In Dina, resistance to ZYMV is conferred by a single recessive gene (Abul Hayja and Al-Shahwan, 1991). In TMG-1, resistances to ZYMV, ZYFV, and MWMV (Provvidenti, 1987; Gilbert-Albertini et al, 1995; Kabelka and Grumet, 1996, respectively) are each conferred by single recessive genes. Resistance to PRSV-W is due to a single dominant, or incompletely dominant, gene (Wai and Grumet, 1995a) while resistance to WMV involves two types of resistances under separate genetic control (Wai and Grumet, 1995b). In addition, the gene conferring resistance to ZYMV in TMG-1 appears to be the same as, or tightly linked to, genes conferring resistance to WMV and MWMV (Wai and Grumet, 1995b; Kabelka and Grumet, 1996).

Since there are two sources of resistance to ZYMV reported in the literature, we sought to examine the relationship between the genes conferring resistance to ZYMV in TMG-1 and Dina, and to test for possible differences in the performance of the resistance alleles. In addition, since a genetic relationship exists between resistance to ZYMV and resistance to other potyviruses in TMG-1, we sought to examine Dina for resistance to other cucurbit potyviruses.

MATERIAL AND METHODS

Cucumber genotypes

The inbred cucumber (*Cucumis sativus* L.) line 'TMG-1', resistant to ZYMV, WMV, PRSV-W, and MWMV (Provvidenti, 1985; Kabelka and Grumet, 1996), was provided by Dr. Jack Staub (USDA, University of Wisconsin at Madison). Progeny of the Dutch hybrid 'Dina', true-breeding for resistance to ZYMV (Abul Hayja and Al-Shahwan, 1991), were initially provided by Dr. Ken Owens (Peto Seed Company, Inc., Saticoy, CA) and then increased by self- or sib-pollination in the greenhouse. The susceptible parental genotype used in this investigation is the open-pollinated cucumber cultivar 'Straight-8' (ST-8; W. Atlee Burpee & Company, Warminster, PA). Progeny of the crosses between Dina and TMG-1, Dina and ST-8, and ST-8 and TMG-1 were produced in the greenhouse. The F_1 progeny of each cross were either self- or sib-pollinated to produce the F_2 generations; backcrosses were made to both parents.

Virus inocula, inoculation procedures, experimental designs and data analysis

Propagation of virus inocula, methods of inoculation, experimental designs, and data analysis were performed as described by Kabelka and Grumet (1996). The relationship between symptom expression and virus levels were examined by enzyme-linked immunosorbent assay (ELISA). The ELISA procedure was performed as described by Wai and Grumet (1995a).

ELISA data was analyzed by analysis of variance of a randomized complete block design with five replicates; individual comparisons were analyzed by orthogonal contrasts. Various ZYMV isolates used to examine the allelic relationship in TMG-1 and Dina include: Connecticut, Taiwan 1, Taiwan 2, Florida, and California, (provided by Dr. R. Provvidenti, Cornell University, Geneva, NY) and the Israeli non-aphid transmissible strain (NAT) (provided by Dr. B. Raccah, The Volcani Institute, Bet Dagan, Israel). Two additional potyviruses used in this investigation were MWMV, provided by Dr. D. Purcifull (University of Florida, Gainesville, FL), and PRSV-W (PV-380; American Type Culture Collection, Rockville, MD).

RESULTS

Relationship between the ZYMV resistance in TMG-1 and Dina

To determine if the resistance genes in TMG-1 and Dina are at the same locus, the progeny of this cross were inoculated at the cotyledon stage with ZYMV. Seven to ten days post inoculation, the susceptible genotype, ST-8, developed a systemic mosaic pattern of the foliage while TMG-1, Dina and their progeny initially appeared symptom-free. However, 14 days post inoculation, the two resistant genotypes, TMG-1 and Dina, exhibited a strikingly different phenotype (Figure 3.1). Dina responded with a distinct veinal chlorosis limited to the first and/or second true leaves. Subsequent leaves in Dina, however, remained symptom-free. In TMG-1, this distinct

Figure 3.1 Phenotypic response of the cucumber lines Dina (left) and TMG-1 (right) 14 days post cotyledon inoculation with ZYMV. Dina responds with a distinct veinal chlorosis limited to the first and/or second true leaves with subsequent leaves symptom-free. In TMG-1, this distinct veinal chlorosis phenotype is not observed.

veinal chlorosis phenotype was not observed. TMG-1 remained symptom-free or would occasionally exhibit sparse veinal chlorosis. For simplicity, hereafter the response to ZYMV inoculation in Dina is referred to as a veinal chlorosis phenotype and in TMG-1 as a symptom-free phenotype.

The distinct veinal chlorosis phenotype in Dina only occurs when cotyledons are inoculated with ZYMV. When inoculated with ZYMV at the second and third true leaf stage, no distinct veinal chlorosis on the inoculated leaves or subsequent leaves was seen (data not shown).

Although Dina and TMG-1 have different phenotypes on the first and/or second true leaves, evaluation of segregation for resistance and susceptibility indicates that the two resistance alleles are at the same locus (Table 3.1). From the third true leaf on, all F_1, F_2 and backcross progeny of TMG-1 and Dina remained symptom-free, vigorous and healthy.

Differences in the performance of the resistance alleles in TMG-1 and Dina

Although progeny from the cross between Dina and TMG-1 were all resistant, there was segregation for the veinal chlorosis phenotype. The veinal chlorosis appeared to be due to a single gene with the veinal chlorosis phenotype dominant to the symptom-free phenotype (Table 3.2). The reciprocal F_1 progeny exhibited the veinal chlorosis phenotype, the reciprocal F_2 progeny segregated in a 1:3 (symptom-free phenotype:veinal chlorosis phenotype) ratio while the backcross to the TMG-1 parent segregated

Table 3.1 Segregation for resistance to ZYMV in the progeny derived from the cucumber lines TMG-1 and Dina.

Parent or progeny	Number of Plants[b]		Expected ratio (R:S)[a]	
	Resistant[c]	Susceptible	If alleles at same locus	If alleles at different loci
TMG-1 (resistant)	32	0		
DINA (resistant)	31	0		
Straight-8 (susceptible)	0	32		
F1(TMG x Dina) (Dina x TMG)	64	0	1:0	0:1
F2(TMG x Dina) (Dina x TMG)	320	0	1:0	7:9
BC (F1 x TMG) (TMG x F1)	160	0	1:0	1:1
BC (F1 x Dina) (Dina x F1)	160	0	1:0	1:1

[a]expected ratios based on inheritance of resistance to ZYMV in TMG-1 as a single recessive gene and in Dina as a single recessive gene.
[b]data pooled from two independent experiments.
[c]in TMG-1, Dina, and their progeny, symptom expression ranged from no observable symptoms (symptom-free) to a distinct veinal chlorosis confined to one or two leaves with subsequent leaves vigorous and healthy.

Table 3.2 Response of the cucumber lines TMG-1, Dina and their progeny 14 days post cotyledon inoculation with ZYMV.

Parent or progeny	Number of plants: Symptom-free[a]	Number of plants: Distinct veinal chlorosis[b]	Expected ratio[c]	X^2
TMG-1	32	0	1:0	
Dina	0	31	0:1	
F1(Dina x TMG) (TMG x Dina)	0	64	0:1	
F2 (Dina x TMG)[d] (TMG x Dina)	90	230	1:3	1.51 ns
BC (F1 x TMG)[e] (TMG x F1)	72	88	1:1	1.40 ns
BC (F1 x Dina)[f] (Dina x F1)	3	157	0:1	0.04 ns

ns, non-significant X^2 value

[a]plants remain either symptom-free or exhibit an occasional sparse veinal chlorosis.

[b]symptom expression of the distinct veinal chlorosis was confined to one or two true leaves with subsequent leaves symptom-free.

[c]expected ratios based on the distinct veinal chlorosis phenotype dominant to the symptom-free phenotype.

[d]data pooled from two independent experiments. Each experiment fits the predicted ratios. X^2exp1 = 1.41 ns; X^2exp2 = 0.21 ns; X^2 homogeneity = 0.32, df = 1

[e]data pooled from two independent experiments. Each experiment fits the predicted ratios. X^2exp1 = 2.82 ns; X^2exp2 = 0.00 ns; X^2 homogeneity = 2.13, df = 1

[f]data pooled from two independent experiments. Each experiment fits the predicted ratios. X^2exp1 = 0.08 ns; X^2exp2 = 0.00 ns; X^2 homogeneity = 0.28, df = 1

1:1 symptom-free phenotype:veinal chlorosis phenotype. Backcrosses to the parental genotype Dina, all exhibited the veinal chlorosis phenotype limited to the first and/or second true leaves.

Possible explanations for the observed inheritance of the veinal chlorosis phenotype are: (1) Dina and TMG-1 have different alleles for resistance at the same locus, one of which causes the veinal chlorosis, or (2) there is a separate locus responsible for the veinal chlorosis phenotype. To distinguish between these possibilities, TMG-1 and Dina were each crossed to a common susceptible genotype, ST-8, and their progeny tested for response to cotyledon inoculation with ZYMV (Table 3.3). TMG-1 remained symptom-free, Dina responded with a veinal chlorosis of the first and/or second true leaves with subsequent leaves symptom-free and the susceptible genotype, ST-8, exhibited a systemic mosaic pattern throughout. Consistent with published results (Provvidenti, 1987; Abul Hayja and Al-Shahwan, 1991), segregation ratios of resistance vs. susceptibility indicate that resistance to ZYMV in both Dina and TMG-1 is conferred by a single recessive gene. Fourteen days post inoculation, individuals derived from the Dina and ST-8 cross developed the veinal chlorosis phenotype while individuals derived from the ST-8 and TMG-1 cross remained symptom-free. If a separate gene was responsible for veinal chlorosis we would expect segregating progeny within a cross to show all three phenotypes of mosaic, veinal chlorosis and symptom-free. This was not the observed. Only one

Table 3.3 Distribution of response in the cucumber lines TMG-1, Dina, Straight-8, and their progeny to inoculation with ZYMV

Parent or Progeny	Resistant		Susceptible	Expected ratios for resistance, susceptibility and distinct veinal chlorosis phenotype in resistant individuals											
				Model 1 Alleles are different and no additional factor for distinct veinal chlorosis phenotype required				An additional factor is responsible for distinct veinal chlorosis phenotype seen in resistant individuals							
								Model 2 If additional factor in ST8 is dominant				Model 3 If additional factor in ST8 is recessive			
	Symptom-free[a]	Distinct veinal chlorosis	Mosaic	symp-free : chlorosis : mosaic $z^Y z^Y$	$z^D z^D$	ZZ	X^2	symp-free : chlorosis : mosaic zzvv	zzV–	Z–V–(vv)	X^2	symp-free : chlorosis : mosaic zzvv	zzV–	Z–V–(vv)	X^2
TMG-1 (T)	40	0	0	1	0	0		1	0	0		1	0	0	
Dina (D)	0	40	0	0	1	0		0	1	0		0	1	0	
Straight-8 (ST8)	0	0	40	0	0	1		0	0	1		0	0	1	
F1 (ST8xT)	0	0	40	0	0	1		0	0	1		0	0	1	
F1 (DxST8)	0	0	40	0	0	1		0	0	1		0	0	1	
F2 (ST8xT)	38	2	120	1	0	3	0.06 ns	1	3	12	100.83**	1	0	3	0.06 ns
F2 (DxST8)	1	73	245	0	1	3	0.60 ns	0	1	3	0.61 ns	1	3	12	19.67**
BC (ST8xT) x ST8	0	0	80	0	0	1		0	0	1		0	0	1	
BC (DxST8) x ST8	0	0	80	0	0	1		0	0	1		0	0	1	
BC (ST8xT) x T	38	0	42	1	0	1	0.11 ns	1	1	2	34.38**	1	0	1	0.11 ns
BC (DxST8) x D	0	43	37	0	1	1	0.31 ns	0	1	1	0.31 ns	0	1	1	0.31 ns

ns, non-significant X^2 value
**, significant X^2 value
[a]plants remain either symptom-free or exhibit an occasional sparse veinal chlorosis

type of resistance phenotype, either veinal chlorosis or symptom-free was observed among the progeny from a given cross. Thus segregation ratios for the veinal chlorosis phenotype support a model in which the resistance alleles in TMG-1 and Dina are performing differently; a separate locus is not responsible for the veinal chlorosis phenotype.

The three parental genotypes were examined to determine whether the different phenotypes reflected differences in virus accumulation (Figure 3.2). Virus titers were measured from the first through the third true leaves post inoculation with ZYMV. TMG-1 remained symptom-free and showed essentially no significant virus accumulation (analysis of variance). Dina exhibited the veinal chlorosis phenotype and significant virus levels in the first and second true leaves. However, subsequent symptom-free leaves, in Dina, revealed no detectable virus accumulation. As expected, ST-8 had high levels of virus accumulation.

The alleles from Dina and TMG-1 were also examined for differences in response to various ZYMV isolates (Table 3.4). None of the isolates (Taiwan 1, Taiwan 2, Florida, NAT and California) overcame the resistance in either TMG-1 or Dina. TMG-1 remained symptom-free in response to all tested isolates; Dina exhibited the veinal chlorosis phenotype of the first and/or second true leaves to all isolates except the California isolate where no symptoms were observed.

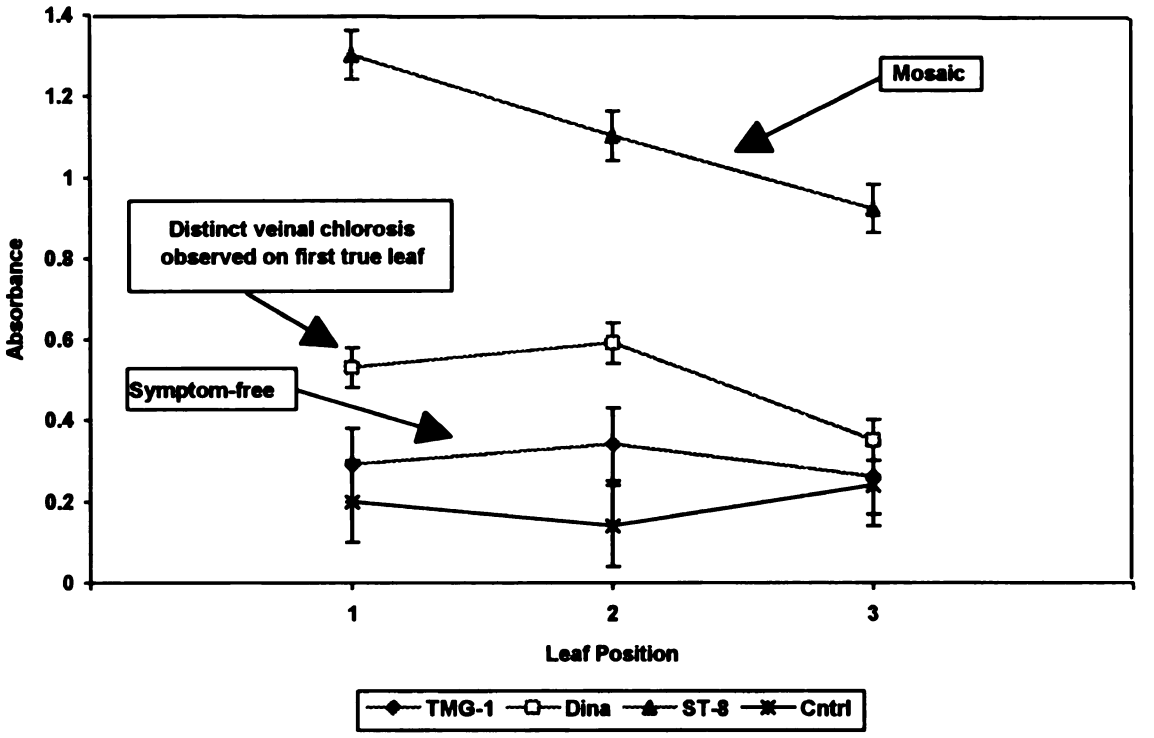

Figure 3.2 Virus accumulation in the cucumber lines TMG-1, Dina and Straight-8 two weeks post cotyledon inoculation with ZYMV. Each point is the mean +/- SE of five replicate plants. Virus levels were measured by ELISA.

Table 3.4 Response of the cucumber lines TMG-1, Dina and Straight-8 to inoculation with several potyviruses

Potyvirus	TMG-1	Dina	Straight-8
ZYMV - Connecticut	Resistant - symptom-free	Resistant - distinct veinal chlorosis phenotype	Susceptible - systemic mosaic
- Taiwan 1	Resistant - symptom-free	Resistant - distinct veinal chlorosis phenotype	Susceptible - systemic mosaic
- Taiwan 2	Resistant - symptom-free	Resistant - distinct veinal chlorosis phenotype	Susceptible - systemic mosaic
- Florida	Resistant - symptom-free	Resistant - distinct veinal chlorosis phenotype	Susceptible - systemic mosaic
- California	Resistant - symptom-free	Resistant - symptom-free	Susceptible - systemic mosaic
MWMV	Resistant - symptom-free	Resistant - symptom-free	Susceptible - systemic rugosity and silvering
PRSV-W	Resistant - symptom-free	Resistant - symptom-free	Susceptible - systemic rugosity

Response of Dina to inoculation with other potyviruses

Previous studies have identified TMG-1 to possess resistance to ZYMV, WMV, PRSV-W and MWMV (Provvidenti, 1985; Kabelka and Grumet, 1996). In addition, the resistance gene for ZYMV, in TMG-1, is the same as, or tightly linked to, the genes for resistance to WMV and MWMV (Wai and Grumet, 1995b; Kabelka and Grumet, 1996). Since Dina possesses resistance to ZYMV, we sought to determine if it is also resistant to other potyviruses and, if so, to examined whether a similar genetic relationship exists between the resistance gene(s). We, therefore, tested Dina for response to PRSV-W and MWMV. Following cotyledon inoculation with PRSV-W and MWMV, Dina remained symptom-free and, interestingly, exhibited no veinal chlorosis of the first and/or second true leaves as seen with ZYMV inoculation (Table 3.4).

The resistance to MWMV in Dina was further examined by characterizing the mode of inheritance and determining its relationship to the ZYMV resistance gene. Following rub inoculation with MWMV, the susceptible genotype, ST-8, showed a mild systemic rugosity and silvering of the true leaves 14 days post inoculation (Table 3.5). Symptom intensity of MWMV varied during the course of plant growth; the rugosity and silvering of the true leaves would fade occasionally but then resurge to full expression. Dina remained symptom-free. The F_1 progeny of Dina and ST-8 were susceptible, the F_2 progeny segregated in a 1:3 [resistant(R):susceptible(S)]

Table 3.5 Response of the cucumber lines Dina, Straight-8 and their progeny to inoculation with MWMV

Parent or progeny	Number of plants		Expected ratio (R:S)[a]	X^2
	Resistant	Susceptible		
Dina (D)	20	0		
Straight-8 (ST8)	0	20		
F1 (DxST8)	0	20	0:1	
F2 (DxST8)	35	92	1:3	0.317 ns
BC (F1xD)	65	73	1:1	0.356 ns
BC (F1xST8)	0	20	0:1	

ns, non-significant X^2 value
[a]expected ratios based on a single recessive gene model, R = resistant, S = susceptible.

ratio while the backcross to the resistant parent segregated in a 1:1 (R:S) ratio. Backcrosses to the susceptible parent were all susceptible. These segregation ratios support a model in which the inheritance of resistance to MWMV in Dina is conferred by a single recessive gene.

Genetic relationship between resistance to ZYMV and resistance to MWMV in Dina

By analogy to ZYMV resistance in TMG-1, one would predict that the gene for MWMV resistance in Dina is the same as, or tightly linked to, the gene for ZYMV resistance. This hypothesis was tested in two ways: (1) sequential inoculation of Dina, ST-8, and their progeny with ZYMV and MWMV and (2) tests for segregation of MWMV resistance in the progeny of Dina and TMG-1.

The sequential inoculation procedure consisted of cotyledon inoculation of Dina, ST-8, and their progeny with ZYMV followed by true leaf inoculation of the resistant individuals with MWMV (Table 3.6). Additional control plants were included to verify successful inoculation at the true leaf stage with MWMV. Symptoms of ZYMV, a systemic mosaic pattern, developed on the susceptible ST-8 plants approximately seven days post cotyledon inoculation. Fourteen days post cotyledon inoculation with ZYMV, all resistant individuals developed veinal chlorosis of the first and/or second true leaves with subsequent leaves symptom-free. The resistant individuals were then sequentially inoculated on the asymptomatic fourth and fifth true

Table 3.6 Response of the cucumber lines Dina, Straight-8 and their progeny to cotyledon inoculation with ZYMV followed by true leaf inoculation of resistant individuals with MWMV.

Parent or progeny	Total plants	Cotyledon inoculation with ZYMV		Sequential inoculation of ZYMV resistant individuals with MWMV at true leaf stage	
		Number of plants		Number of plants	
		Resistant	Susceptible	Resistant	Susceptible
Dina (D)	20	20	0	20	0
Straight-8 (ST8)[a]	10	0	10	--	--
Straight-8 (ST8)[b]	10	--	--	0	10
F1(DxST8)[a]	10	0	10	--	--
F1(DxST8)[b]	10	--	--	0	10
F2 (DxST8)[c]	320	75	245	75	0
BC (DxST8)xD[d]	80	38	42	38	0

[a]control plants to verify successful inoculation at cotyledon stage with ZYMV.
[b]control plants to verify successful inoculation at true leaf stage with MWMV.
[c]data fits the expected and predicted segregation ratios based on resistance to ZYMV conferred by a single recessive gene. $X^2 = 0.33$ (non-significant X^2 value)
[d]data fits the expected and predicted segregation ratios based on resistance to ZYMV conferred by a single recessive gene. $X^2 = 0.12$ (non-significant X^2 value)

leaves with MWMV (18 days post cotyledon inoculation with ZYMV). Symptoms of MWMV developed approximately 14 days post true leaf inoculation on the susceptible genotype, ST-8, and consisted of mild rugosity and silvering of the true leaves. In all cases, those individuals resistant to cotyledon inoculation with ZYMV, although initially responding with a veinal chlorosis limited to the first and/or second true leaves, remained free of symptoms upon true leaf inoculation with MWMV.

The second approach tested for segregation of MWMV resistance among the progeny of Dina and TMG-1 (Table 3.7). Fourteen days post inoculation the susceptible genotype, ST-8, developed a mild rugosity and silvering of the true leaves while the resistant genotypes TMG-1 and Dina remained symptom-free throughout. Evaluation of segregation for resistance and susceptibility in the progeny of TMG-1 and Dina indicates that the two MWMV resistance alleles are located at the same locus as all individuals of the F_1, F_2 and backcross generations remained symptom-free.

DISCUSSION

Segregation ratios among the F_1, F_2 and backcross progeny of TMG-1 and Dina indicate that the ZYMV resistance alleles are at the same locus. However, the two sources of ZYMV resistance seem to perform differently. Dina responds to ZYMV cotyledon inoculation with a distinct veinal chlorosis of the first and/or second true leaves with subsequent leaves symptom-free.

Table 3.7 Segregation for resistance to MWMV in the progeny derived from the cucumber lines TMG-1 and Dina.

Parent or progeny	Number of Plants		Expected ratio (R:S)[a]	
	Resistant	Susceptible	If alleles at same locus	If alleles at different loci
TMG-1 (resistant)	20	0		
Dina (resistant)	20	0		
Straight-8 (susceptible)	0	20		
F1(TMG x Dina) (Dina x TMG)	20	0	1:0	0:1
F2(TMG x Dina) (Dina x TMG)	140	0	1:0	7:9
BC (F1 x TMG) (TMG x F1)	80	0	1:0	1:1
BC (F1 x Dina) (Dina x F1)	80	0	1:0	1:1

[a]expected ratios based on inheritance of resistance to MWMV in TMG-1 as a single recessive gene and in Dina as a single recessive gene.

The progeny of TMG-1 and Dina segregated for the observed distinct veinal chlorosis phenotype which was dominant to the symptom-free phenotype. An alternate possibility, that an additional factor was responsible for the distinct veinal chlorosis phenotype, was ruled out based on evaluation of the segregating progeny of TMG-1 and Dina crossed to a common susceptible background.

The symptoms in Dina appear also to reflect virus replication and movement. Consistent with previous studies (Wai and Grumet, 1995a; Al-Shahwan et al, 1995), detectable virus, limited to the first and second true leaves, was observed in Dina while no detection of virus accumulation was present in TMG-1. The mechanism in Dina that limits virus to the first and second true leaves is unknown. However, studies to examine the mechanism of resistance to ZYMV in Dina are currently in progress.

The distinct veinal chlorosis phenotype in Dina only occurs when cotyledons are inoculated with ZYMV. When inoculated with ZYMV at the second and third true leaf stage, no distinct veinal chlorosis on the inoculated leaves or subsequent leaves was seen. This suggests a tissue-specific expression of the resistances (cotyledon vs. true leaf) to ZYMV in Dina. Potyvirus resistance exhibiting a tissue-specific expression has been observed previously in cucurbits. In TMG-1, resistance to WMV is conferred by two independently segregating factors; one resistance is expressed in the cotyledons and throughout the plant while the second resistance is expressed

only in true leave tissue (Wai and Grumet, 1995b). Tissue specificity was also observed in muskmelon cultivars resistant to PRSV-W (Gibb et al, 1994). One resistant cultivar 'Cinco' exhibited only occasional mild systemic symptoms on cotyledon inoculated plants. The hybrid of Cinco crossed to a susceptible cultivar 'Planter's Jumbo' exhibited mild systemic symptoms upon cotyledon inoculation but remained symptom-free upon true leaf inoculation.

From the data obtained, it appears that a series of alleles occur at the *zym* gene locus and that these alleles differ in their effectiveness and dominance relationships. Both of the resistance alleles are recessive to the wild type susceptible allele (Zym^+). The veinal chlorosis phenotype associated with the resistance allele from Dina (zym^{Dina}) is dominant to the symptom-free phenotype associated with the resistance allele from TMG-1 (zym^{TMG-1}). Therefore, at the *zym* locus, $Zym^+ > zym^{Dina} > zym^{TMG-1}$. Of the two resistances examined, however, the allele from TMG-1 would represent the most effective resistance as no distinct veinal chlorosis phenotype was seen and no accumulation of virus was evident. Possibly, the relative effectiveness of the different alleles at the *zym* gene locus is proportional to the extent by which they reduce virus multiplication or prevent movement.

Series of alleles occurring at specific loci and differing in their effectiveness have been described previously in literature. For example, in *Capsicum*, response to infection by tobacco mosaic virus (TMV) was found to

be controlled by a series of alleles where *L* provides localization of TMV, L^i provides "imperfect" localization of TMV and L^+ causes mottling (Holmes, 1937). In tomato, three resistance factors, *Tm-1*, *Tm-2* and $Tm\text{-}2^2$ confer resistance to TMV (Hall, 1980; Stobbs and MacNeill, 1980). Of the three, *Tm-2* and $Tm\text{-}2^2$ are considered allelic. Both *Tm-2* and $Tm\text{-}2^2$ can induce a hypersensitive response to TMV but $Tm\text{-}2^2$ is described as a more effective allele as the hypersensitive response is more extreme. In addition, TMV strains 1 and 2 overcome *Tm-2* but not $Tm\text{-}2^2$.

As mentioned earlier, previous studies have identified TMG-1 to possess resistance to several potyviruses including ZYMV, WMV, PRSV-W and MWMV (Provvidenti, 1985; Kabelka and Grumet, 1996). In addition, the resistance gene for ZYMV, in TMG-1, appears to be the same as, or tightly linked to, the genes for resistance to WMV and MWMV (Wai and Grumet, 1995b; Kabelka and Grumet, 1996). Our studies reveal that, like TMG-1, Dina also possesses resistance to multiple potyviruses including PRSV-W and MWMV. Interestingly, however, upon inoculation with PRSV-W or MWMV, Dina remains symptom-free; not exhibiting the veinal chlorosis phenotype as seen with several isolates of ZYMV except the California isolate. Although MWMV, PRSV-W and ZYMV-California are distinct potyviruses, one shared feature is the time interval preceding observable symptoms in the susceptible genotype. While symptoms of most ZYMV isolates developed approximately 7-10 days post inoculation symptoms of

MWMV, PRSV-W and ZYMV-California did not become evident until approximately 14-21 days post inoculation. Possibly this difference in time of onset represents a slower rate of virus multiplication or movement resulting in lack of veinal chlorosis in Dina. Possibly it represents a difference in resistance mechanisms. As virus accumulation was observed in the first and second true leaves of Dina post inoculation with ZYMV-Connecticut, it would be interesting to determine if a lack of veinal chlorosis represents a lack of virus accumulation. It is possible, however, to have virus accumulation in the absence of symptoms. Previous studies with TMG-1 and PRSV-W (in contrast to ZYMV) have indicated that although TMG-1 is symptom-free post inoculation with PRSV-W, there is PRSV-W accumulation (Wai and Grumet, 1995a).

The resistance to MWMV in Dina was further examined by characterizing the mode of resistance and determining its relationship to the ZYMV resistance gene. Resistance to MWMV in Dina, like resistance to ZYMV, is conferred by a single recessive gene. Sequential inoculation of progeny possessing resistance to ZYMV followed by MWMV suggests that both resistances are conferred by the same gene, or two tightly linked genes. In addition, tests for segregation of MWMV resistance in the progeny of Dina and TMG-1 indicate the two MWMV resistance alleles are at the same locus.

Collectively, analysis of data suggests that one gene, or two tightly linked genes, confer resistance to ZYMV and MWMV in both TMG-1 and

Dina. Tests of allelism suggest the genes for resistance found in TMG-1 and Dina are at the same locus. However, as evidenced by Dina's strikingly different response to inoculation with ZYMV, both with regard to symptom expression, virus accumulation and dominance relationships, the ZYMV resistance alleles in TMG-1 and Dina are likely to be different indicating that a series of alleles exist at the *zym* locus where $Zym^{+} > zym^{Dina} > zym^{TMG\text{-}1}$. Since the differences in response were observed only for ZYMV and not MWMV it is possible that rather than a single gene conferring resistance to all three potyviruses, the MWMV, WMV and ZYMV genes may be part of a gene cluster for potyvirus resistance in cucumber.

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CHAPTER 4

Characterization of genes controlling resistance to the watermelon strain of papaya ringspot virus in cucumber: allelism and cosegregation with other potyviruses

ABSTRACT

The watermelon strain of papaya ringspot virus (PRSV-W) is one of several potyviruses causing damage to cucurbit production worldwide. Within the cucumber germplasm, sources of resistance to PRSV-W have been identified in 'Surinam', a cultivar from South America, and 'Taichung Mou Gua' (TMG-1), a single plant selection from a Chinese cultivar from Taiwan. Each resistance is due to a single gene. Although resistance from Surinam is recessive, and the source from TMG-1 is incompletely dominant, segregation ratios among the progeny of TMG-1 and Surinam indicate that the resistances to PRSV-W are at the same locus. The different dominance relationships, which are exhibited even when crossed to a common susceptible parent, may indicate different alleles or the influence of other modifying factors. Unlike Surinam, TMG-1 also possesses resistances to several other potyviruses all of which appear to be at the same locus, or tightly-linked loci. This study indicated that resistance to PRSV-W in TMG-1 is also at the same locus, or tightly-linked to ZYMV resistance.

INTRODUCTION

The watermelon strain of papaya ringspot virus (PRSV-W) is one of several potyviruses causing damage to cucurbit production worldwide (Purcifull et al, 1984b). Other cucurbit potyviruses include zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV) and the Moroccan watermelon mosaic virus (MWMV) (Lisa and Lecoq, 1984; Purcifull et al, 1984a; McKern et al, 1993). In cucumber (*Cucumis sativus* L.), Provvidenti (1985) identified two accessions, 'Surinam', a cultivar from South America, and 'Taichung Mou Gua' (TMG-1), a single plant selection from a Chinese cultivar from Taiwan, as sources of resistance to PRSV-W. The inheritance of resistance to PRSV-W in both Surinam and TMG-1 have been characterized. In the cultivar Surinam, resistance to PRSV-W is conferred by a single recessive gene (Wang et al, 1984). In TMG-1, segregation ratios indicated that resistance to PRSV-W is conditioned by a single dominant gene. However, the effectiveness of this resistance varied at times suggesting either incomplete dominance or the involvement of other genetic or environmental factors (Wai and Grumet, 1995a).

Although Surinam only possesses resistance to PRSV-W, TMG, also is resistant to ZYMV, WMV, MWMV and zucchini yellow fleck virus (ZYFV) (Provvidenti, 1985; Gilbert-Albertini et al, 1995; Kabelka and Grumet, 1996a). Resistance to ZYMV is conferred by a single recessive gene (Provvidenti, 1987), as is resistance to ZYFV and MWMV (Gilbert-Albertini

et al, 1995; Kabelka and Grumet, 1996a) while resistance to WMV involves two types of resistances under separate genetic control (Wai and Grumet, 1995b). In addition, the gene conferring resistance to ZYMV appears to be the same as, or tightly linked to, genes conferring resistance to WMV and MWMV (Wai and Grumet, 1995b; Kabelka and Grumet, 1996a).

Since there are two sources of resistance to PRSV-W with reported differences in dominance relationships, we sought to examine the allelic and dominance relationships between the gene(s) conferring resistance to PRSV-W in TMG-1 and Surinam and to compare performance when crossed to a common susceptible parent. In addition, since the gene conferring resistance to ZYMV in TMG-1 appears to be the same as, or tightly linked to, the genes conferring resistance to WMV and MWMV we sought to determine if a similar relationship exists between resistance to ZYMV and resistance to PRSV-W in TMG-1.

MATERIAL AND METHODS

Cucumber genotypes

The inbred cucumber (*Cucumis sativus* L.) lines 'TMG-1', resistant to ZYMV, WMV, PRSV-W and MWMV (Provvidenti, 1985; Kabelka and Grumet, 1996a) and 'Surinam Local', resistant to PRSV-W (Wang et al, 1984), were provided by Dr. Jack Staub (USDA, University of Wisconsin at Madison) and Dr. R. Provvidenti (Cornell University, Geneva, NY),

respectively. The two susceptible parental genotypes used in this investigation were the inbred lines 'Wisconsin-2757' (WI-2757; Peterson et al, 1982; provided by Dr. Jack Staub) and 'Straight-8' (ST-8; W. Atlee Burpee and Company, Warminster, PA). Progeny of the crosses between TMG-1 and Surinam, ST-8 and Surinam, and ST-8 and TMG-1, were produced in the greenhouse. The F_1 progeny of each cross were self-pollinated to produce the F_2 generation or backcrossed to both parents. Production of the F_1, F_2 and backcross progeny of WI-2757 and TMG-1 was described by Wai and Grumet (1995a). F_3 families, used to analyze the relationship between resistance to ZYMV and PRSV-W, were produced by first screening 1,479 F_2 (WI-2757 x TMG-1) individuals for resistance to ZYMV in the greenhouse. Three-hundred-seventy-three ZYMV resistant F_2 individuals [3:1 (S:R); $X^2 = 0.08$, non-significant] were then transplanted to the field. As WI-2757 is gynoecious (dominant trait; Peterson et al, 1982), monoecious individuals were identified and self-pollinated by hand under controlled conditions. Fifty-one F_3 families were produced.

Virus inocula, inoculation procedures, experimental designs and data analysis

PRSV-W (PV-380; American Type Culture Collection, Rockville, MD) and ZYMV (Connecticut Strain) were propagated in *Cucurbita pepo* L. cvs. 'Black Beauty' (SeedWay Inc., Elizabethtown, PA) or 'Midas' (Willhite Seed, Poolville, TX). Propagation of virus inocula, methods of inoculation,

experimental designs, and data analysis were performed as described by Kabelka and Grumet (1996a).

RESULTS

Relationship between the PRSV-W resistances in TMG-1 and Surinam

To determine if the resistance genes in TMG-1 and Surinam are at the same locus, the progeny of this cross were inoculated with PRSV-W. Approximately 14-21 days post inoculation, the susceptible genotype ST-8 developed a systemic rugosity of the foliage while the resistant genotypes, TMG-1 and Surinam, remained symptom-free (Table 4.1). Evaluation of segregation for resistance and susceptibility in the progeny of TMG-1 and Surinam indicated that the two resistance alleles are at the same locus as all individuals of the F_1, F_2 and backcross generations remained free of symptoms.

Differences in the performance of the resistance alleles in TMG-1 and Surinam

Progeny analysis of TMG-1 and Surinam indicated that the resistance genes are at the same locus, however, one is reported to be recessive (Wang et al, 1984) and the other dominant, or incompletely dominant (Wai and Grumet, 1995a). Since the reported inheritance studies for TMG-1 and Surinam were performed with different susceptible lines, a direct comparison of their dominance relationships could not be made. Therefore, in this study,

Table 4.1 Segregation for resistance to PRSV-W in the progeny derived from the cucumber lines TMG-1 and Surinam.

Parent or Progeny	No. Plants[a]	
	Resistant	Susceptible
TMG-1 (resistant)	16	0
Surinam (resistant)	16	0
Straight-8 (susceptible)	0	16
F1 (TMGxSurinam)	16	0
F2	320	0
BC (F1xTMG) (TMGxF1)	80	0
BC (F1xSurinam) (SurinamxF1)	200	0

[a]data pooled from two independent experiments.

TMG-1 and Surinam were each crossed to a common susceptible genotype (ST-8) and their progeny tested to further examine the dominance relationships of each allele. In addition, since the inheritance of resistance to PRSV-W was initially characterized utilizing WI-2757 and TMG-1, their progeny were included for comparative purposes.

Consistent with previous studies (Wang et al, 1984; Wai and Grumet, 1995a), segregation ratios indicated that the inheritance of resistance to PRSV-W in Surinam is conferred by a single recessive gene and in TMG-1 by a single incompletely dominant gene (Table 4.2). Plants were scored visually when symptoms were optimally expressed, approximately 14-21 days post inoculation. Following rub inoculation with PRSV-W, the resistant genotypes, TMG-1 and Surinam, remained free of symptoms. Symptom expression on the susceptible genotype ST-8 was a systemic rugosity while the susceptible genotype WI-2757 exhibited a systemic silver banding and/or rugosity. The F_1 progeny of ST-8 and Surinam were susceptible exhibiting symptoms throughout the plant that were as severe as the susceptible parent. The F_2 progeny segregated in a 1:3 (R:S) ratio while the backcross to the resistant parent segregated in a 1:1 (R:S) ratio. Progeny backcrossed to the susceptible parent were all susceptible.

The F_1 progeny of TMG-1 crossed with WI-2757 or ST-8, however, exhibited an intermediate phenotype where the young leaves and growing points were symptom-free but the older leaves exhibited rugosity and/or

Table 4.2 Response of the cucumber lines TMG-1, Surinam, Straight-8, WI-2757 and their progeny to inoculation with PRSV-W

Parent or progeny	Number of Plants			Expected ratios[d]	X^2
	Resistant[a]	Intermediate[b]	Susceptible[c]		
TMG-1	15	0	0		
Surinam (S)	15	0	0		
Straight-8 (ST8)	0	0	15		
WI-2757 (2757)	0	0	15		
F1 (2757xT)	0	20	0		
F1 (ST8xT)	0	20	0		
F1 (ST8xS)	0	0	40		
F2(2757xT)	14	29	15	1:2:1	0.04 ns
F2(ST8xT)	33	55	32	1:2:1	0.85 ns
F2(ST8xS)	33	0	86	1:3	0.33 ns
BC (2757xT)xT	21	19	0	1:1	0.04 ns
BC (ST8xT)xT	28	32	0	1:1	0.46 ns
BC (ST8xS)xS	32	0	28	1:1	0.04 ns
BC (ST8xT)xST8	0	29	31	1:1	0.04 ns
BC 2757x(2757xT)	0	17	23	1:1	0.62 ns
BC (ST8xS)xST8	0	0	60	1:1	0.00 ns

ns, non-significant X^2 value.
[a]plants symptom-free, vigorous and healthy.
[b]young leaves and growing points remain symptom-free but older leaves exhibit rugosity and/or silver banding.
[c]systemic rugosity and/or silver banding throughout the plant, similar to that exhibited by either susceptible genotype.
[d]expected ratios based on inheritance of resistance to PRSV-W in TMG-1 as an incompletely dominant gene and in Surinam as a single recessive gene.

silver banding. The F_2 progeny segregated in a 1:2:1 [resistant(R):intermediate(I):susceptible(S)] ratio. Progeny backcrossed to the resistant parent segregated in a 1:1 (R:I) ratio while progeny backcrossed to the susceptible parent segregated in a 1:1 (I:S) ratio. It should be noted, however, that while the young leaves and growing points of individuals exhibiting the intermediate phenotype remained symptom-free, the rugosity and/or silver banding of the older leaves varied from nearly symptom-free to severe. Although symptom severity of the intermediate phenotype varied with environment, it did not appear to be affected by the different susceptible parents. When the progeny of ST-8 x TMG-1 and WI-2757 x TMG-1 were grown side-by-side, the symptom severity of the intermediate phenotype was equivalent.

Genetic relationship between resistance to ZYMV and resistance to PRSV-W in TMG-1

Earlier studies have identified sources of resistance in cucumber to several potyviruses. As mentioned earlier, TMG possesses resistance to PRSV-W, ZYMV, WMV, ZYFV, and MWMV (Provvidenti, 1985; Gilbert-Albertini et al, 1995; Kabelka and Grumet, 1996a) while the Dutch hybrid Dina is resistant to ZYMV, PRSV-W, and MWMV (Abul Hayja and Al-Shahwan, 1991; Kabelka and Grumet, 1996b). In addition, the alleles for ZYMV, WMV and MWMV resistance in TMG-1 and the alleles for ZYMV and MWMV resistance in Dina appear to be at the same locus, or tightly

linked loci (Wai and Grumet, 1995b; Kabelka and Grumet, 1996b). The relationship of PRSV-W to the other potyviruses, however, is not known. Interestingly, unlike TMG-1 and Dina which are resistant to multiple potyviruses including PRSV-W, Surinam is susceptible to ZYMV, WMV (Wang et al, 1984), and MWMV (data not shown). As would be expected due to the recessive inheritance of resistance to ZYMV (Provvidenti, 1987) the F_1 progeny of TMG-1 and Surinam which are resistant to PRSV-W are susceptible to ZYMV (data not shown).

To examine the relationship of PRSV-W resistance to the other potyvirus resistances, we tested for association between ZYMV and PRSV-W. To test this, two approaches were taken. The first approach was sequential inoculation of backcross progeny of (ST-8 x TMG-1) x TMG-1. This procedure consisted of cotyledon inoculation with PRSV-W followed by true leaf inoculation of the resistant individuals with ZYMV (Table 4.3A). In a separate experiment, this procedure was reversed, the cotyledons were inoculated with ZYMV and true leaves of the resistant individuals were inoculated with PRSV-W (Table 4.3B). In all experiments, additional control plants were included to verify successful inoculation at the true leaf stage. Symptoms of PRSV-W in the susceptible genotype, ST-8, were as described earlier. Symptoms of ZYMV, a systemic mosaic pattern of the true leaves, developed on the susceptible genotype, ST-8, approximately seven days post cotyledon or true leaf inoculation. The resistant genotype, TMG-1, remained

Table 4.3 **Response of TMG-1, Straight-8, the F_1 and backcross progeny to sequential inoculation with ZYMV and PRSV-W.**

A. Cotyledon inoculation with PRSV-W followed by true leaf inoculation of resistant individuals with ZYMV.

Parent or progeny	Total plants	Cotyledon inoculation with PRSV-W: Number of plants			Sequential inoculation of PRSV-W resistant individuals with ZYMV at true leaf stage: Number of plants	
		Resistant	Intermediate[a]	Susceptible	Resistant	Susceptible
TMG-1 (T)	10	10	0	0	10	0
Straight-8 (ST8)[b]	10	0	0	10	--	--
Straight-8 (ST8)[c]	5	--	--	--	0	5
F1(ST8xT)[b]	10	0	10	0	--	--
F1(ST8xT)[c]	5	--	--	--	0	5
BC (ST8xT)xT[d]	160	77	83	0	77	0

[a]young leaves and growing points remain symptom-free but older leaves exhibit rugosity and/or silver banding
[b]control plants to verify successful inoculation at cotyledon stage with PRSV-W
[c]control plants to verify successful inoculation at true leaf stage with ZYMV
[d]data fits the expected and predicted segregation ratios based on resistance to PRSV-W conferred by an incompletely dominant gene. $X^2 = 0.16$ (non-significant X^2 value)

B. Cotyledon inoculation with ZYMV followed by true leaf inoculation of resistant individuals with PRSV-W.

Parent or progeny	Total plants	Cotyledon inoculation with ZYMV: Number of plants		Sequential inoculation of ZYMV resistant individuals with PRSV-W at true leaf stage: Number of plants		
		Resistant	Susceptible	Resistant	Intermediate[a]	Susceptible
TMG-1 (T)	10	10	0	10	0	0
Straight-8 (ST8)[b]	10	0	10	--	--	--
Straight-8 (ST8)[c]	5	--	--	0	0	5
F1(ST8xT)[b]	10	0	10	--	--	--
F1(ST8xT)[c]	5	--	--	0	5	0
BC (ST8xT)xT[d]	160	78	82	78	0	0

[a]young leaves and growing points remain symptom-free but older leaves exhibit rugosity and/or silver banding
[b]control plants to verify successful inoculation at cotyledon stage with ZYMV
[c]control plants to verify successful inoculation at true leaf stage with PRSV-W
[d]data fits the expected and predicted segregation ratios based on resistance to ZYMV conferred by a single recessive gene. $X^2 = 0.06$ (non-significant X^2 value)

free of symptoms to both PRSV-W and ZYMV inoculation. In all cases, those individuals resistant to cotyledon inoculation with the first virus remained symptom-free upon true leaf inoculation with the second virus.

The second approach to evaluate whether a relationship exists between the resistance to ZYMV and resistance to PRSV-W was to screen F_3 families that were produced by self-pollination of F_2 individuals selected for resistance to ZYMV. Progeny from each ZYMV-resistant F_2 individual were inoculated either with PRSV-W or ZYMV (Table 4.4). As expected, all families were resistant to ZYMV. If the two resistances had been segregating independently, 9/16th of the F_3 families should be susceptible or segregating for susceptibility to PRSV-W. There was, however, no segregation for susceptibility to PRSV-W either within or among the F_3 families screened. These F_3 families previously had been screened for response to MWMV and all were resistant (Kabelka and Grumet, 1996a).

DISCUSSION

Segregation ratios among the progeny of TMG-1 x Surinam, ST-8 (susceptible genotype) x TMG-1, and ST-8 x Surinam, indicated that the resistances to PRSV-W are at the same locus but may be due to different alleles. The source of resistance from Surinam is recessive whereas the source from TMG-1 is incompletely dominant. While three distinct classes of response (resistant, intermediate, and susceptible) were observed in the

Table 4.4 PRSV-W and ZYMV inoculation of F_3 families homozygous recessive at the *zym* locus derived from the inbred cucumber lines TMG-1 and WI-2757. F3 families were produced by self-pollinating F_2 individuals selected for resistance to ZYMV.

Potyvirus	No. F3 families[a] showing resistance	No. F3 families susceptible or segregating for resistance
PRSV-W	51	0
ZYMV	51	0

[a]tests of F_3 families included ten individuals/family

segregating population of TMG-1 crossed with WI-2757 or ST-8, the intermediate phenotype varied; the young leaves and growing points remained symptom-free but the older leaves exhibited various degrees of rugosity and/or silver banding ranging from nearly symptom-free to severe. Previous studies evaluating the inheritance of resistance to PRSV-W in TMG-1 (utilizing WI-2757 as a susceptible genotype) describe resistance as a single dominant gene (Wai and Grumet, 1996a). However, full expression of resistance in the F_1 progeny depended on optimal growing conditions. When grown in cool weather or low light levels, occasional mild symptoms were observed. Although the resistance to PRSV-W in TMG-1 appeared incompletely dominant in this study, possibly suggesting a series of alleles at the *Prsv-2* locus, the existence of modifying and/or environmental factors influencing the effectiveness of the resistance gene in TMG-1 cannot be ruled out. In fact, a combination of factors may be involved.

Judgment as to whether a single locus resistance is dominant or incompletely dominant is typically based on phenotypic evaluation of the F_1 and segregating progeny of the cross between pure-breeding resistant and susceptible parental lines for symptoms of virus infection post inoculation with a particular virus. This subjective observation of phenotypic symptom expression can be misleading. An example of this is found in sugarbeet which possesses resistance to beet mosaic virus (Lewellen, 1973). Evaluation of the parental and segregating populations, at an early stage of infection

(10-14 days post virus inoculation) fell into discrete 3:1 (R:S) classes for the F_2 progeny and 1:0 (R:S) for the progeny backcrossed to the resistant parent. However, at a later stage of infection (20-30 days post virus inoculation) and depending on the time of year and greenhouse temperatures, the F_2 progeny segregated as a 1:2:1 (R:I:S) ratio and the backcross to the resistant parent segregated as a 1:1 (R:I) ratio. Another example is found in common bean which possesses resistance to soybean mosaic virus governed by an incompletely dominant gene (Kyle and Provvidenti, 1993). In the homozygous state, no detectable systemic symptoms were observed but virus could be detected in inoculated leaves. In the heterozygous state, under field or summer greenhouse conditions, individual plants remained vigorous, or only slightly stunted, and develop local chlorosis with systemic symptoms consisting of a mild to moderate mottling. However, this incompletely dominant gene will appear completely dominant when plants are inoculated and grown under winter greenhouse conditions. A third example is resistance to tobacco mosaic virus in tomato (Fraser and Loughlin, 1980). In the heterozygous state, the resistance allele appears completely dominant with no viral symptoms observed. However, measurement of virus levels of the heterozygote revealed virus replication suggesting incomplete dominance or gene dosage-dependence.

Effectiveness of resistance also may be influenced by the genetic background of the host cultivar. An example of this is found in barley which

possesses tolerance to barley yellow dwarf virus (BYDV) (Jones and Catherall, 1970). Reduced effectiveness of tolerance, conferred by an incompletely dominant gene was observed with slow growth rate considered either the result of host genetic factors or environmental conditions. Interestingly, the effectiveness of tolerance was recovered when introduced into rapidly growing genotypes. In a follow-up study, Catherall, et al (1970), evaluated five gene donor varieties that showed different levels of tolerance to BYDV. The one variety showing the greatest tolerance was the fastest growing while the least tolerant was the slowest growing variety. Interestingly, TMG-1 is a fast growing cultivar; this feature may be influencing the apparent effectiveness of the resistance gene(s).

In addition to the growth rate component of BYDV resistance, there may also be allelic differences. When the tolerance factors from the BYDV resistant varieties were introduced into similar genetic backgrounds, the tolerance factor from the fastest growing variety consistently provided the highest tolerance suggesting a series of alleles occurring at the BYDV locus differing in their effectiveness and dominance relationships.

Earlier studies have identified sources of resistance in cucumber to several potyviruses. TMG possesses resistance to PRSV-W, ZYMV, WMV, ZYFV, and MWMV (Provvidenti, 1985; Gilbert-Albertini et al, 1995; Kabelka and Grumet, 1996a) and the Dutch hybrid Dina possesses resistance to PRSV-W, ZYMV and MWMV (Abul Hayja and Al-Shahwan, 1991; Kabelka

and Grumet 1996a). In addition, the alleles for ZYMV, WMV and MWMV resistance in TMG-1 and the alleles for ZYMV and MWMV resistance in Dina appear to be at the same locus, or tightly linked loci (Wai and Grumet, 1995b; Kabelka and Grumet, 1996a,b). In this study, an association between resistance to PRSV-W and resistance to ZYMV has been identified in TMG-1. Sequential inoculation of ZYMV-resistant backcross progeny with PRSV-W (or PRSV-W-resistant backcross progeny with ZYMV), and analysis of F_3 families derived from F_2 individuals selected for resistance to ZYMV, indicate that both resistances are conferred by the same gene, or tightly linked genes (less than 1 cM; product-ratio method). These results are also consistent with the observation that both the PRSV-W and ZYMV resistances are linked to the *bi* locus for bitterfree cotyledons (Wai et al, 1996).

The existence of simply inherited genes, or clusters of separate tightly-linked genes, that confer resistance to two or more distinct potyviruses have been described previously in several species. For example, resistance to WMV and bean yellow mosaic virus is conferred by a single recessive gene in *Pisum sativum* (Schroeder and Provvidenti, 1971), and in *Cucurbita moschata*, a single dominant gene confers resistance to both ZYMV and WMV (Gilbert-Albertini et al, 1993). In *Phaseolus vulgaris*, the possibility of a single gene, or cluster of tightly linked genes cosegregating as a unit with the *I* gene, conditions resistance and/or lethal necrosis to a set of nine potyviruses including bean common mosaic virus, WMV, blackeye cowpea

mosaic virus, cowpea aphid-borne mosaic virus, azuki bean mosaic virus, Thailand Passiflora virus, soybean mosaic virus, passionfruit woodiness virus-K and ZYMV. (Kyle and Dickson, 1988; Fisher and Kyle, 1994). In *Pisum sativum*, well defined clusters of tightly-linked loci conferring resistance to a total of 11 potyviruses are located on two chromosomes (Provvidenti and Hampton, 1993; Provvidenti and Niblett, 1994). Chromosome 2 contains resistance to WMV, bean yellow mosaic virus, pea mosaic virus, the NL-8 strain of bean common mosaic virus, the lentil strain of pea seed-borne mosaic virus, clover yellow vein virus, and the Australian strain of passionfruit woodiness virus while chromosome 6 includes resistance to three pathotypes of pea seed-borne mosaic virus, clover yellow vein virus, and white lupin mosaic virus.

Despite the inability to break the linkage among the resistances to PRSV-W, ZYMV, WMV, and MWMV, in TMG-1, these resistances may not all be due to a single gene. Lines of evidence suggesting that the resistances to multiple potyviruses in cucumber may be conferred by a cluster of tightly linked genes rather than a single gene include: (1) Resistance to PRSV-W in TMG-1 appears incompletely dominant while resistance to ZYMV is recessive. It is possible that these differences may be due to varying effectiveness of one gene against different potyviruses, but it is also possible that they are due to two different genes. (2) The mechanisms of resistance to PRSV-W and ZYMV in TMG-1 also appear to be different. Previous studies

indicated that when TMG-1 was inoculated with PRSV-W, high virus levels were detected despite a lack of symptoms, however, little or no virus was detected when TMG-1 was inoculated with ZYMV (Wai and Grumet, 1995a). (3) Dina, which is resistant to ZYMV, PRSV-W and MWMV shows different responses to ZYMV than to PRSV-W or MWMV inoculation. A distinct veinal chlorosis phenotype limited to the first and second true leaves was observed with ZYMV cotyledon inoculation whereas no symptoms were observed with PRSV-W or MWMV inoculation (Kabelka and Grumet, 1996b). (4) Although Surinam is resistant to PRSV-W, it is susceptible to ZYMV, WMV and MWMV. Again this may be due to different responses of one gene to different potyviruses or it may be that Surinam only possesses one member of a gene cluster. As mentioned earlier, although resistance to PRSV-W in TMG-1 appeared incompletely dominant, the existence of modifying and/or environmental factors influencing the effectiveness of the resistance gene in TMG-1 could not be ruled out. Possibly the effectiveness of PRSV-W resistance in TMG-1 is influenced other members of a cluster of resistance genes.

In conclusion, although we have not been able to break the linkage associations among the resistances to PRSV-W, ZYMV, WMV, and MWMV in TMG-1, varying responses with regard to dominance relationships, resistance mechanisms, symptom expression, and which viruses are protected against,

support the possibility that multiple potyvirus resistance in cucumber is conferred by a tightly-linked cluster of resistance genes.

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SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

Cucumbers and other cucurbit crops are subject to severe losses due to an array of potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W) and the Moroccan watermelon mosaic virus (MWMV). Sources of resistance to several of these viruses have been identified and characterized within the cucumber (*Cucumis sativus* L.) germplasm. An inbred line derived from the Chinese cucumber cultivar 'Taichung Mou Gua' (TMG-1) possesses resistance to ZYMV, WMV and PRSV-W. The Dutch hybrid 'Dina' possesses resistance to ZYMV and the cultivar 'Surinam Local' is resistant to PRSV-W. This study demonstrated TMG-1 also possesses resistance to MWMV and that this resistance is conferred by a single recessive gene. Sequential inoculation of progeny possessing resistance to ZYMV followed by MWMV (or MWMV followed by ZYMV) and analysis of F_3 families derived from F_2 individuals selected for resistance to ZYMV indicate that both resistances are conferred by the same, or two tightly linked genes. Earlier work with TMG-1 indicated that the recessive gene for ZYMV resistance also conferred resistance to WMV. Therefore it appears that a

single recessive gene, or cluster of recessive genes, in TMG-1, governs resistance to at least three potyviruses, ZYMV, WMV and MWMV.

Tests of allelism indicate that the genes for resistance to ZYMV in TMG-1 and Dina are at the same locus. However, they exhibit different phenotypes in response to cotyledon inoculation with ZYMV. Dina exhibits a distinct veinal chlorosis and accumulation of virus limited to the first and second true leaves whereas TMG-1 is symptom-free and lacks virus accumulation. The distinct veinal chlorosis phenotype was dominant to the symptom-free phenotype and shown not to be due to an additional separate factor. Therefore, at the *zym* locus, a series of alleles occur differing in their effectiveness and dominance relationships with $Zym^{+} > zym^{Dina} > zym^{TMG\text{-}1}$.

Our studies also reveal that in addition to ZYMV resistance, Dina possesses resistance to PRSV-W and MWMV and that the gene for MWMV resistance is at the same locus as the ZYMV resistance. In addition, tests of allelism indicate the gene for resistance to MWMV in TMG-1 and Dina are at the same locus. In contrast to ZYMV inoculation, however, Dina does not exhibit the distinct veinal chlorosis phenotype when inoculated with PRSV-W or MWMV. Since the differences in response were observed only for ZYMV and not MWMV, it is possible that rather than a single gene conferring resistance to ZYMV, WMV and MWMV, the resistances may be part of a gene cluster for potyvirus resistance in cucumber.

Segregation ratios among the progeny of TMG-1 and Surinam, and TMG-1 and Surinam crossed with a common susceptible parent, indicate the sources of resistance to PRSV-W are at the same locus but different dominance relationships indicate either different alleles or the effect of other modifying factors as resistance to PRSV-W in Surinam is recessive while in TMG-1 it is incompletely dominant.

Although Surinam only possesses resistance to PRSV-W, TMG-1 possesses resistance to ZYMV, WMV, MWMV and PRSV-W. In addition, an association between resistance to ZYMV and resistances to WMV and MWMV was found in TMG-1. Our studies also indicated that in addition to the tight linkage between resistances to ZYMV, WMV, and MWMV in TMG-1, resistance to PRSV-W also cosegregated with resistance to ZYMV. Sequential inoculation of ZYMV-resistant backcross progeny followed by PRSV-W (or PRSV-W resistant backcross progeny followed by ZYMV) and analysis of F_3 families derived from F_2 individual selected for resistance to ZYMV, indicate that both resistances are conferred by the same gene, or tightly-linked genes. Therefore, it appears that a single gene, or cluster of tightly-linked genes, governs resistance to ZYMV, WMV, MWMV and PRSV-W in TMG-1.

Despite the inability to break the linkage among the resistances to ZYMV, WMV, MWMV and PRSV-W in TMG-1, these resistances may not all be due to a single gene. Several lines of evidence suggest that the resistances

to multiple potyviruses in cucumber may be conferred by a cluster of tightly-linked genes: (1) the mode of inheritance of resistance to PRSV-W in TMG-1 appears incompletely dominant while to ZYMV it is recessive, (2) the mechanisms of resistance with regards to viral accumulation also appears to be different for PRSV-W vs. ZYMV in TMG-1, (3) Dina, which is resistant to ZYMV, PRSV-W and MWMV shows different responses to inoculation with these potyviruses, and (4) although Surinam is resistant to PRSV-W, it is susceptible to ZYMV, WMV and MWMV. It is possible that these differences may be due to varying effectiveness of one gene against different potyviruses, but it is also possible that they are due to a cluster of potyvirus resistance genes.

In conclusion, although we have not been able to break the linkage associations among the resistances to PRSV-W, ZYMV, WMV, and MWMV in TMG-1, varying responses with regard to dominance relationships, resistance mechanisms, symptom expression, and which viruses are protected against, support the possibility that multiple potyvirus resistance in cucumber is conferred by a tightly-linked cluster of resistance genes.

Several additional experiments can be performed to further investigate the relationship between the potyvirus resistance genes in cucumber. Segregation ratios among the F_1, F_2 and backcross progeny of TMG-1 and Dina indicated that the ZYMV resistance alleles are at the same locus, however, the two sources of ZYMV resistance seem to perform differently.

Dina responds to ZYMV cotyledon inoculation with a distinct veinal chlorosis phenotype and accumulation of virus limited to the first and second true leaves whereas TMG-1 remains symptom-free and lacks virus accumulation. The distinct veinal chlorosis phenotype in Dina only occurs when cotyledons are inoculated. When inoculated with ZYMV at the true leaf stage, no distinct veinal chlorosis was seen. The mechanism in Dina that limits virus to the first and second true leaves is unknown, however, studies to examine the mechanism of resistance to ZYMV in Dina are currently in progress utilizing a tissue-printing method.

Our studies also indicated that in addition to ZYMV resistance Dina possesses resistance to PRSV-W and MWMV. However, upon inoculation with PRSV-W or MWMV, Dina remains symptom-free not exhibiting the distinct veinal chlorosis phenotype as seen with several isolates of ZYMV except the California isolate. Although MWMV, PRSV-W and ZYMV-California are distinct potyviruses, one shared feature is the time interval preceding observable symptoms in the susceptible genotype. While symptoms of most ZYMV isolates developed approximately 7-10 days post inoculation symptoms of MWMV, PRSV-W and ZYMV-California did not become evident until approximately 14-21 days post inoculation. Possibly this difference in time of onset represents a slower rate of virus multiplication or movement resulting in lack of veinal chlorosis in Dina. Possibly it represents a difference in resistance mechanisms. It would be

interesting to determine if a lack of veinal chlorosis represents a lack of virus accumulation. ELISA and/or tissue-printing can be used to address these questions.

As mentioned, Dina also possesses resistance to PRSV-W. The inheritance of resistance to PRSV-W in Dina was not characterized. In addition, previous studies with TMG-1 and PRSV-W (in contrast to ZYMV) have indicated that although TMG-1 is symptom-free post inoculation with PRSV-W, there is virus accumulation. Therefore, it would be interesting to compare the resistances to PRSV-W found in TMG-1 and Dina.

APPENDICES

APPENDIX A

APPENDIX A

Linkage relationship of potyvirus resistance to bitterfree cotyledons

To further understand the relationship of potyvirus resistances to each other, linkage relationships were investigated. In Surinam and in TMG-1 the gene conferring resistance to PRSV-W is reported to be linked with bitterfree cotyledons (Wang et al, 1987; Pierce and Wehner, 1990; Wai et al, 1996). Since the gene conferring resistance to PRSV-W appears to be the same as, or tightly linked to, genes conferring resistance to ZYMV in TMG-1, we tested for cosegregation between resistance to ZYMV and the bitterfree gene. For comparison we included tests for cosegregation between resistance to PRSV-W and the bitterfree gene.

The inbred cucumber (*Cucumis sativus* L.) 'TMG-1', resistant to ZYMV, WMV, PRSV-W, MWMV (Provvidenti, 1985; Kabelka and Grumet, 1996) and the susceptible parental genotype 'Wisconsin 2757 (WI-2757; Peterson et al, 1982) were provided by Dr. Jack Staub (USDA, University of Wisconsin at Madison). Linkage tests between potyvirus resistance genes and the bitterfree gene were performed using the F_2 progeny of WI-2757 and TMG-1. Production of this progeny is as described by Wai and Grumet (1995). Propagation of virus inocula, methods of inoculation, and experimental designs were performed as described by Kabelka and Grumet

(1996). The presence of cucurbitacins (bitterness) was assayed by tasting a small piece of the cotyledons (DeCosta and Jones, 1971). Linkage relationships and distances were analyzed using Chi square, the product-ratio method, and Linkage 1 version 3.5 (Suiter et al, 1983).

WI-2757 is bitterfree (*bibi*) while TMG-1 and the F_1 progeny of this cross are bitter (*Bi*--). Goodness-of-fit tests show that resistance to PRSV-W, resistance to ZYMV, and bitterfree cotyledons fit expected ratios for single gene traits (Table A.1). When traits were examined in pairwise combination for linkage relationships there were variable results (Table A.2). Resistance to ZYMV appears to be linked to the bitterfree gene when evaluating a large population (n=157; estimated map distance 21.3 cM) but independently segregating when evaluating a smaller population (n=80). Pooled data mapped to a distance of 24.8 cM. When considering resistance to PRSV-W as a single incompletely dominant trait, linkage to the bitterfree gene, when evaluating a large population (n=149), mapped to a distance of 37.2 cM but segregated independently when evaluating a small population (n=79). Pooled data (n=228) mapped to a distance of 46.4 cM. In previous reports, the gene conferring resistance to PRSV-W was considered as a single dominant trait and linked to the bitterfree gene at an approximate map distance of 28 cM (Wai et al, 1996). Interestingly, evaluating the current data as a single dominant trait brings the map distance between resistance to PRSV-W and the bitterfree gene closer to the previous estimated map

distance in literature. When evaluating the large population (n=149), linkage of PRSV-W resistance and the bitterfree gene mapped to a distance of 29.9 cM. However, the smaller population (n=79) still segregated independently. Pooled data mapped to a distance of 35.5 cM.

It is possible that in order to accurately evaluate either the ZYMV or the PRSV-W resistances to bitterfree cotyledons, a large population is necessary. This conclusion is not unique and has been observed for other groups of associated traits in cucumber such as andromonoecious sex expression, protruding ovary, spine size and frequency, red mature fruit, heavy netting of fruit, and black or brown spines (Pierce and Wehner, 1990). Further experiments will be required to clarify these differing results.

Table A.1 Single trait goodness of fit tests for characters analyzed in the F_2 progeny of WI-2757 x TMG-1 cucumber.

	Allele	Expected Ratio[a]	Observed Ratio	Observed Phenotype[b]	X^2
Experiment 1	*bi*	3:1	115:45	Bi:bi	0.68 ns
	zym	3:1	115:42	S:R	0.17 ns
	bi	3:1	103:46	Bi:bi	2.44 ns
	Prsv-2	1:2:1	32:76:41	S:I:R	1.15 ns
Experiment 2	*bi*	3:1	57:23	Bi:bi	0.42 ns
	zym	3:1	58:22	S:R	0.15 ns
	bi	3:1	59:21	Bi:bi	0.02 ns
	Prsv-2	1:2:1	23:32:24	S:I:R	2.86 ns
Pooled data	*bi*	3:1	172:68	Bi:bi	1.25 ns
	zym	3:1	173:64	S:R	0.40 ns
	bi	3:1	162:67	Bi:bi	2.00 ns
	Prsv-2	1:2:1	55:108:65	S:I:R	1.51 ns

ns, non-significant X^2 value

[a]expected ratios based on bitterfree inherited as a single recessive trait in WI-2757, ZYMV resistance conferred by a single recessive gene in TMG-1, and resistance to PRSV-W conferred by an incompletely dominant gene in TMG-1.

[b]Bi: bitter; bi: bitterfree; S: susceptible; R: resistant; I: intermediate phenotype

Table A.2 Tests for co-segregation of pairs of alleles. Phenotypic classes and their frequencies in F_2 progeny derived from matings between the WI-2757xTMG-1 cucumber lines.

Loci *bi*[a] *zym*[b]	Total plants	Number of plants (X-Y-)	(X-yy) (TMG-1)	(xxY-) (WI-2757)	(xxyy)	X^2 (9:3:3:1)	Estimated map distance
Experiment 1	157	73	40	42	2	18.1**	21.3 cM
Experiment 2	80	37	20	21	2	7.3 ns	
Pooled data	237	110	60	63	4	25.3**	24.8 cM

Loci *bi*[a] *Prsv-2*[c]	Total plants	Number of plants (X-YY) (TMG-1)	(X-Yy)	(X-yy)	(xxYY)	(xxYy)	(xxyy) (WI-2757)	X^2 (3:6:3:1:2:1)	Estimated map distance
Experiment 1	149	26	59	18	6	17	23	25.5**	37.2 cM
Experiment 2	79	19	22	17	4	10	7	4.5 ns	
Pooled data	228	45	81	35	10	27	30	20.5**	46.4 cM

Table A.2 (continued)

Loci			Number of plants					
bi [a]	Prsv-2 [d]	Total plants	(X-Y-) (TMG-1)	(X-yy)	(xxY-)	(xxyy) (WI-2757)	X^2 (9:3:3:1)	Estimated map distance
Experiment 1		85	85	18	23	23	24.6**	29.9 cM
Experiment 2		79	41	17	14	7	1.5 ns	
Pooled data		228	126	35	37	30	19.6**	35.5 cM

**, significant X^2 value
ns, non-significant X^2 value
[a]bitterfree is inherited as a single recessive trait in WI-2757.
[b]ZYMV resistance in TMG-1 is conferred by a single recessive gene.
[c]calculated X^2 and estimated map distance based on PRSV-W resistance in TMG-1 considered a single incompletely dominant gene.
[d]calculated X^2 and estimated map distance based on PRSV-W resistance in TMG-1 considered a single dominant gene.

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